



Biogas and Bioethanol Production in Organic Farming

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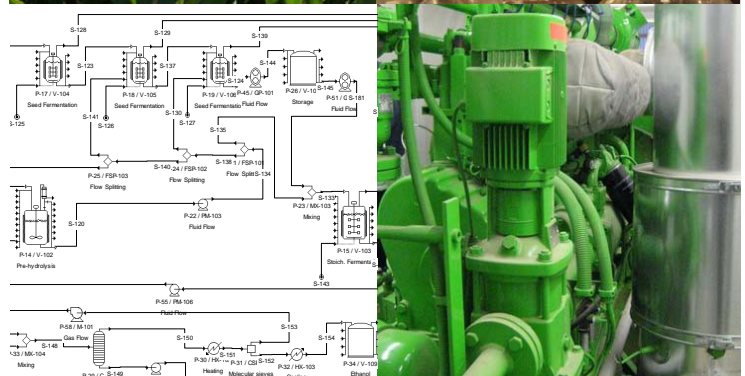
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Biogas and Bioethanol Production in Organic Farming

Risø-PhD-Report

Piotr Oleskowicz-Popiel
Risø-PhD-64(EN)
August 2010

Risø DTU
National Laboratory for Sustainable Energy



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Biogas and Bioethanol Production in Organic Farming

PhD Thesis

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Submitted: August 2010

Bioenergy and Biorefinery Program

Biosystems Division

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Preface

The presented PhD project was carried out at Bioenergy and Biorefinery Program, Biosystems Division, National Laboratory for Sustainable Energy – Technical University of Denmark (Risø DTU) from September 2007 to August 2010 under supervision of Head of Program Jens Ejbye Schmidt and co-supervision of Senior Scientist Anne Belinda Thomsen. The project also involves 3 months of research carried out during my external stay at Lawrence Berkeley National Laboratory, where I worked in the Joint BioEnergy Institute under supervision of Professor Harvey Blanch.

The thesis consists of two parts. First one is an introduction providing background information on organic farming, ethanol and anaerobic digestion processes, and concept of on-farm bioenergy production. Second part consists of the following papers:

Paper I: Oleskowicz-Popiel P., Thomsen M.H., Nielsen H.B., Schmidt J.E., Thomsen A.B.: Characterization of most relevant feedstock for biogas and bioethanol production in organic farming. Vth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunisia, 25-28 May, 2008.

Paper II: Oleskowicz-Popiel P., Nielsen H.B., Thomsen A.B., Schmidt J.E.: Biogas and ethanol potentials in selected biomasses for organic farming. *Submitted*

Paper III: Oleskowicz-Popiel P., Schmidt J.E., Thomsen A.B.: Ensiling – a wet-storage and a biological pretreatment method for bio-ethanol production from lignocellulosic biomasses. *Submitted*

Paper IV: Oleskowicz-Popiel P., Lehtinen T.M., Schmidt J.E., Thomsen A.B.: Ensiling – wet-storage and pretreatment of corn stover to produce bioethanol. *Submitted*

Paper V: Christensen A.D., Kadar Z., Oleskowicz-Popiel P., Thomsen M.H.: Production of bioethanol from organic whey using *Kluyveromyces marxianus*. Journal of Industrial Microbiology and Biotechnology, 2010, DOI 10.1007/s10295-010-0771-0.

Paper VI: Oleskowicz-Popiel P., Thomsen M.H., Thomsen A.B., Schmidt J.E.: A simulation model of combined biogas, bioethanol and protein fodder co-production in organic farming. International Journal of Chemical Reactor Engineering, 2009, vol.7, Article A71.

Paper VII: Oleskowicz-Popiel P., Schmidt J.E.: Techno-economic analysis of bioethanol and biogas production in organic farming. *Proceeding submitted to 12th World Congress on Anaerobic Digestion*, Guadalajara, Mexico, October 31st – November 4th, 2010.

Paper VIII: Klein-Marcuschamer D., Oleskowicz-Popiel P., Simmons B.A., Blanch H.W.: Techno-economic analysis of biofuels: a wiki-based platform for lignocellulosic biorefineries. Biomass and Bioenergy, 2010, doi:10.1016/j.biombioe.2010.07.033.

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I would like also to thank Jens Bo Holm-Nielsen, my supervisor during my Master thesis and my previous boss at Aalborg University and University of Southern Denmark, for inspiring me and introducing me to the green energy world and consequently inspiring me to start PhD studies in this area.

Finally, I would like to thank my mom and my dad for their never-ending believes in me and for support in all my decisions. I also would like to thank my sister and her family for cheering as well as backing me up anytime I needed.

Last but not least, I would like to thank my girlfriend Magda for her infinite believe in me and for bringing even more happiness to my life.

Abstract

The consumer demand for environmentally friendly, chemical free and healthy products, as well as concern regarding industrial agriculture's effect on the environment has led to a significant growth of organic farming. On the other hand, organic farmers are becoming interested in direct on-farm energy production which would lead them to independency from fossil fuels and decrease the greenhouse gas emissions from the farm. In the presented work, the idea of biogas and bioenergy production at the organic farm is investigated. This thesis is devoted to evaluate such a possibility, starting from the characterization of raw materials, through optimizing new processes and solutions and finally evaluating the whole on-farm biorefinery concept with the help of a simulation software.

At first, different raw materials available at the Danish organic farm were selected and characterized for their methane and ethanol potentials, namely: fresh maize, ensiled maize, fresh rye, ensiled rye, dry rye, fresh clover, clover silage, dry clover, dried vetch, whey permeate and cattle manure. Anaerobic digestion batch experiments were carried out resulting in maize and rye silage demonstrating the highest methane potential. Additionally, continuous anaerobic digestion trials showed that methane yield in the co-digestion of cattle manure and maize silage resulted in a 36% increase of methane production with 33% from maize silage in the feedstock.

The ethanol potential of all the selected materials was estimated based on the sugar amount, resulting in the highest for dry rye and fresh maize. Furthermore, ensiling as a wet-storage and a biological pretreatment method for lignocellulosic ethanol production was investigated. Promising laboratory result were found, concluding that ensiling can maintain the "freshness" of the crop and prevent spoilage of wet-biomass while at the same time having a positive impact on ethanol fermentation process. Enzymatic convertibility tests showed that 51.5%, 36.5%, and 41.9% of the cellulose was converted by cellulytic enzymes in ensiled maize, rye, and clover grass, respectively. Noticeable amounts of ethanol were produced from only ensiled crops, the ethanol production was 33.9%, 28.5%, and 36.9% (by *K.marxianus*) and 30.6%, 28.1% and 34.5% (by *S.cerevisiae*); the yields significantly increased after hydrothermal pretreatment: 79.0%, 74.6%, and 80.2% (by *K.marxianus*) and 72.7%, 81.3% and 76.2% (by *S.cerevisiae*) of the theoretical ethanol yield based on the C6 sugar contents in untreated silage of maize, rye, and clover grass, respectively. It is concluded that ensiling has a high potential as a combined wet-storage and pretreatment method for investigated crops (maize, rye and clover). Additionally, trials with dry agricultural by-product were carried out. Ensiling of corn stover resulted in increased ethanol: 23.1 % compared to 16.4% of the theoretical in ensiled and non-ensiled corn stover, respectively.

Laboratory experiments on ethanol production from organic whey by *K.marxianus* were carried out. This process is planned to be part of the development of a concept for a decentralized biorefinery. It shows that no pasteurization or freezing of whey is necessary and it can be fermented with a high ethanol yield (~0.50 g EtOH/g lactose), and that during continuous fermentation using Ca-alginate-immobilized *K.marxianus*

high ethanol productivity is achieved: 2.5-4.5 g/L/h at dilution rate 0.2/h. This confirmed that *K.marxianus* is suitable for ethanol production from whey as a nutritious and additional carbon source.

The final part of the work was devoted to evaluating the whole concept of the on-farm biorefinery. Within the presented work, a simulation model of on-farm bioenergy production was built. It was calculated that to supply a 100 ha organic farm with energy, 16.2 ha of rye and 14 milking cows is needed to produce ethanol from rye grains and whey. Alternatively, 5.7 ha clover grass, 2.5 ha maize and 13 cows are needed to produce the required biogas from clover silage, maize silage and cattle manure. After the further development of the simulation model, a techno-economic model was built for 5 different scenarios for bioenergy production at 1000 ha organic farm: scenarios Biogas I/II (10%/20% clover grass silage and cattle manure). Scenarios Bioethanol I/II (10%/20% rye grains and whey) and a combination of both (called: Combined). Combined scenario was characterized by the highest investment (3,330,000 USD) and the largest energy produced (29244 GJ/year). Biogas II was second best (26409 GJ/year) and it was characterized by lower investment cost (1,963,000 USD) when compared to the Biogas I which produced (19970 GJ/year) with an investment cost of (2,016,000 USD). Scenarios Bioethanol I and Bioethanol II represented the lowest investment costs (1,115,000 USD and 1,047,000 USD, respectively) and generated the least energy (4034 GJ/year and 5610 GJ/year, respectively). In all scenarios, there was enough fuel produced to supply the farm with self-produced energy.

Finally, an open access modeling tool of lignocellulosic ethanol biorefinery for broad biofuel community was built. Its purpose is to make it possible to analyze, explore and communicate the progress of biofuels production and to make it able to revise it by the academic and professional research community. Overall, it should help to bring the development of lignocellulosic biorefineries closer to reality.

Resumé

Forbruger efterspørgsel på miljøvenlige, kemikaliefrie og sunde produkter, samt bekymring for den miljø påvirkning konventionelt landbrug medfører, har givet anledning til en betydelig vækst i økologisk landbrug. Samtidig har økologiske landmænd i højere grad fået interesse i udviklingen af energiproduktion direkte på bedriften, som kan føre til uafhængighed af fossile brændstoffer og reduktion af drivhusgasemissionen fra bedriften. I det fremlagte arbejde er idéen om biogas og bioenergi produktion i økologisk landbrug undersøgt. Med denne afhandling vurderes en sådan mulighed, begyndende med karakterisering af råvarer i økologisk landbrug, efterfulgt af en optimering af nye processer og løsninger, og til sidst med en evaluering af det decentrale bioraffinaderi-koncept, ved hjælp af simulationssoftware.

Først blev der udvalgt forskellige råvarer fra det økologiske landbrug i Danmark og disse blev karakteriseret i henhold til deres metan- og ethanolpotentialer. De valgte råvarerne var frisk majs, ensileret majs, frisk rug, ensileret rug, tørret rug, frisk kløver, kløverensilage, tørret kløver, tørret vikke, valle samt kvæggylle. Batch eksperimenter af anaerob udrådning viste at majs- og rugensilage havde det største metanpotentiale. Derudover viste forsøg med kontinuert anaerob udrådning, en stigning i metanudbyttet på 36% når gylle blev iblandet med 33% majsensilage.

Ethanolpotentialiet i alle de udvalgte råvarer blev evalueret på baggrund af sukkerindhold, dette resulterede i højest potentiale for tørret rug og frisk majs. Derudover blev det undersøgt hvorvidt ensilering kan fungere som både opbevaringsmetode og som biologisk forbehandling til ethanolproduktion fra lignocellulosisk biomasse. Resultaterne var lovende og det konkluderes at ensilering forebygger nedbrydning (forrådnings) af biomassen og har samtidig en positiv indvirkning på ethanolfermentering. Enzymatisk konvertibilitetstests viste, at 51,5%, 36,5% og 41,9% af cellulose blev konverteret med cellulaseenzymer i henholdsvis ensileret majs, rug og kløvergræs. Mærkbare mængder ethanol blev produceret direkte fra ensilerede afgrøder og udbyttet steg betydeligt efter hydrotermisk forbehandling. Ethanolproduktion direkte efter ensilering var hhv. 33,9%, 28,5%, og 36,9% (ved *K. marxianus*) og 30,6%, 28,1% og 34,5% (ved *S. cerevisiae*) af det teoretiske udbytte baseret på C6 sukkerindhold i ensileret majs, rug og kløvergræs. Efter hydrotermisk forbehandling steg de udbyttet til hhv. 79,0%, 74,6% og 80,2% (ved *K. marxianus*) og 72,7%, 81,3% og 76,2% (ved *S. cerevisiae*). Det konkluderes at ensilering har et højt potentiale som kombineret opbevarings- og forbehandlings- metode for de undersøgte afgrøder (majs, rug og kløver). Desuden blev der foretaget studier på halm, som er et biprodukt i landbruget. Ensilering af majshalm resulterede i en øget ethanol produktion fra 16,4% til 23,1%, sammenlignet med ikke-ensileres majshalm.

Laboratorieundersøgelser af ethanol produktion fra økologisk valle med *K. marxianus* blev gennemført som en delproces til udviklingen af et koncept for et decentralt bioraffinaderi. Forsøgene viser at pasteurisering eller frysning af vallen ikke er nødvendig før fermentering, og at det kan fermenteres med højt ethanoludbytte (~ 0,50 g EtOH/g laktose). Under kontinuert fermentering nåede udbyttet op på 2,5 - 4,5 g/L/t ved

fortyndings hastighed $0,2 \text{ time}^{-1}$, ved hjælp af Ca-alginat-immobiliserede *K. marxianus*. Dette bekræftede, at *K. marxianus* er egnet til ethanol produktion med valle som næringsstof tilskud og ekstra kulstofkilde.

Den sidste del af arbejdet var helliget til at vurdere hele konceptet for decentrale bioraffinaderier direkte på bedriften. Dette arbejde bestod i at opbygge en simulationsmodel for forskellige scenarier ved hjælp af computersoftware. Herigennem blev det beregnet, at for at forsyne en 100ha økologisk gård med energi, skal der eksempelvis bruges 16.2ha rug og 14 malkekøer, hvorfra der produceres ethanol fra rugkerner og valle. Alternativt kan der bruges 5.7ha kløvergræs, 2,5ha majs og 13 kreaturer, hvorfra der producerer biogas fra blandingen af kløverensilage, majsensilage og husdyrgødning. Efter yderligere udvikling af simuleringsmodellen, blev der opstillet en teknisk-økonomisk model som beskrev 5 forskellige scenarier for bioenergi produktionen på en 1000ha økologisk gård. De fem scenarier bestod i Biogas I og II (hhv. 10% og 20% kløvergræsensilage og husdyrgødning), Bioethanol I og II (hhv. 10% / 20% rugkerner og valle) samt kombinationen af biogas og bioethanol (kaldet: Kombineret). Kombineret scenariet gav den største investering (3.330.000 USD), men samtidig mest produceret energi (29.244 GJ/år). Biogas II var næstbedst på energi produktion (26.409 GJ/år), og gav samtidig lavere investeringsomkostninger (1.963.000 USD) i forhold til Biogas I som gav (19.970 GJ/år) for en investering på (2.016.000 USD). Scenarierne Bioethanol I og Bioethanol II gav de laveste investeringsomkostninger (hhv. 1.115.000 og 1.047.000 USD), men genererede også mindst energi (hhv. 4.034 og 5610 GJ/år). I alle scenarier blev der produceret nok brændstof til at forsyne bedrifterne med energi. Slutteligt blev der konstrueret et softwarebaseret modelværktøj med åben adgang, til modellering af ethanolbioraffinaderier fra lignocellulosisk biomasse. Modellens formål er at gøre det muligt at analysere, udforske og kommunikere udviklingen indenfor produktion af biobrændstoffer, og dermed bidrage til at bringe udviklingen af lignocellulosiske bioraffinaderier tættere på reel implementering i samfundet.

Table of Contents

1. INTRODUCTION	1
1.1 OUTLINE OF THE THESIS	2
2. ORGANIC FARMING AND BIOCONCENS CONCEPT	3
3. BIOMASS AND BIOENERGY	7
3.1. RAW MATERIALS	8
3.2. ETHANOL.....	11
3.2.1. <i>Ethanol potential</i>	11
3.2.2. <i>Pretreatment</i>	12
3.2.3. <i>Ethanol fermentation</i>	14
3.3. BIOGAS	16
3.3.1. <i>Process principles</i>	16
3.3.2. <i>Biogas potential</i>	17
3.3.3. <i>Continuous trials</i>	19
3.4. ETHANOL AND BIOGAS CO-PRODUCTION	19
4. BIOREFINERY MODELING	21
4.1. PRINCIPLES	21
4.2. ON-FARM ENERGY PRODUCTION	21
4.3. LIGNOCELLULOSIC BIOREFINERY	29
4.3.1. <i>Techno-economic models</i>	31
5. CONCLUDING REMARKS	33
6. FUTURE OUTLOOK	34
REFERENCES	35
LIST OF PAPERS	45

1. Introduction

There is a concept that the two most important trends on which human society is/will be focused on in the near future are: “a transition toward a modern society based on sustainable resources” and “a technological revolution resulting from advances in understanding (...) living systems” (Lynd et al., 1999).

The organic movement fits well into the vision of such a modern society. Organic farming is becoming more popular and it is gaining more and more customers (Macilwain, 2004). The consumer demand for environmentally friendly, chemical free and healthy products, as well as concern regarding industrial agriculture’s effect on the environment has led to a significant growth of organic farming, especially in Europe and North America (Rigby et al., 2001; Hermansen et al., 2004).

A new concept within the organic movement is the idea of on-farm “green” energy production. Direct on-farm energy production can help to solve two major problems: firstly- the dependency on fossil fuels by the production of fuel and electricity generation from local raw materials; and secondly - reducing GHG emissions by using renewable resources. There is no doubt that global reserves of fossil fuels are depleting and that “peak oil” already has or it is going to be reached during the coming decades. Consequently, within a few decades, the world will begin to run short of its oil supply (Kerr, 2005). Additionally, according to the IPCC report from 2007 (IPCC, 2007): “greenhouse-gases (GHG) emission due to human activities has grown since pre-industrial times. (...) Carbon dioxide is the most important anthropogenic GHG; its annual emission grew by about 80% between 1970 and 2004”. Transport is responsible for 13.1% and energy supply for 25.9% within the total GHG emissions (data from 2004), amounting to 39% (IPCC, 2007).

In order to reduce carbon dioxide emissions, the development of a low energy input agricultural system would help (Daalgard et al., 2000). One possibility could be direct on-farm energy production at the organic farming system. Similar to ecosystems, where diversity of organisms brings stability, the energy supply should be diverse where different technologies co-exist according to surroundings (Logan, 2006). Several modern and environmentally friendly technologies could be used, such as wind, solar, geothermal. This work focuses on energy from biomass, particularly on two technologies: ethanol fermentation and anaerobic digestion. Both bioethanol and biogas could be directly produced on a farm and support organic agriculture with self-produced “green” energy. To establish on-farm energy production, identification and consequently biogas and bioethanol potential of possible raw materials available on the organic farm is necessary. Biomethane potentials studies were discussed in (Angelidaki and Sanders, 2004; Hansen et al., 2004; Angelidaki et al., 2009; Cropgen, 2010) but no raw materials originating from organic farming were identified. Ethanol, on the other hand, is produced only from sugars present in biomass; full potential is measured by total sugars determination (Foyle et al., 2007). In the case of lignocellulosic materials, a pretreatment step is needed (Schmidt and Thomsen, 1998; Yang and Wyman, 2008) prior to practical ethanol potential. Due to variety of pretreatment method, the

potential will differ depending on the applied techniques; moreover, the chosen process always depends on the type of raw material and there is no “one suit all” technology which can be applied. A pretreatment step is crucial for second generation (lignocellulosic) ethanol production (Aden and Foust, 2009) and an optimal, low-tech and low energy demanding process for small scale plant is still needed. Finally, after choosing suitable raw materials and technologies, techno-economic analysis is often a great help before establishing such an on-farm biorefinery (Wingren et al., 2003; Aden and Foust, 2009).

1.1 Outline of the thesis

The main objective of the thesis is to evaluate on-farm bioenergy production in organic agriculture. **Chapter 2** gives an overview of the organic farming agriculture movement and its main principles; furthermore presenting an idea for biomass based renewable energy production to be implemented at the organic farm. **Chapter 3** identifies available raw materials and focuses on two possible technologies: bioethanol and biogas production with new process concepts. Several raw materials available at the organic farm are evaluated for their biogas and bioethanol potentials in **Papers I** and **II**. Evaluation of an ensiling process which could be also a new, low energy demanding pretreatment method for ethanol production is investigated and described in **Papers III** and **IV**. Additionally, **Chapter 3** describes the possible integration of two processes (bioethanol and biogas) in the form of a farm-scale biorefinery. Laboratory trials on the production of bioethanol were carried out and results are presented in **Paper V**. **Chapter 4** goes more into detail of the organic farm biorefinery concept, where it is evaluated from a technological and economical point of view. The design and evaluation of the entire on-farm biorefinery was developed and it is described in **Paper VI** and the results from techno-economic analysis are shown in **Paper VII**. **Paper VIII** presents a process model for lignocellulosic ethanol biorefinery, which is an open tool for biofuel community to help the development of economical and environmentally sustainable biorefineries. At the end, the concluding remarks are drawn in **Chapter 5** and future perspective are presented in **Chapter 6**.

2. Organic farming and BioConcens concept

The definition of organic agriculture formed by the International Federation of Organic Agriculture Movements (IFOAM, 2010) says: "Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved." Following that, the four main principles of organic farming were formed, namely: the principle of health, ecology, fairness, and care (IFOAM, 2010). According to IFOAM, the principles are defined as follows:

- Principle of health – *organic agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible*; in other words, the health of individuals cannot be separated from the health of ecosystems, health is the wholeness and integrity of living systems;
- Principle of ecology – *organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them*; that principle connects organic agriculture with living ecosystems, the production should be based on ecological process and recycling; organic farming should fit the cycles and ecological balances in nature
- Principle of fairness – *organic agriculture should be build on relationships that ensure fairness with regard to the common environment and life opportunities*; it underlines that fairness should be ensured at all levels and to all parties (farmers, workers, consumers, etc.); the principle also insists on providing animals with conditions according to their physiology, natural behavior and well-being;
- Principle of care – *organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment*; increasing efficiency and productivity should not risk health or well-being, therefore new technologies must be assessed; organic farming should prevent significant risk by adopting appropriate technologies and rejecting unpredictable ones.

All of this should help to build more sustainable agriculture production. From these core concepts, a new one has grown: the modern trend in organic farming to become self-sufficient in energy supply. The European Directive does suggest lowering the environmental impact from food production, but it does not directly specify the required usage of renewable energy (EC 2007). On the other hand, sustainable energy sources (as locally produced or recycled organic materials) are of interest for organic farmers. Wood et al. (2006) indicated that the transition to organic farming could reduce greenhouse gas emission and energy use. Gundogmus (2006) compared energy use in conventional and organic farming. Using the example of apricot production in Turkey, it is showed that the total energy requirement is lower using organic farming when compared to the conventional one. This is mostly due higher energy efficiency in organic farming and no use of mineral fertilizer (it has the highest energy input use). The total energy input use was 38% lower for organic production; comprehending the lower yields in organic

farming. The benefit-cost ratios were nearly the same on both production systems (Gundogmus, 2006). Dalgaard et al. (2001) presented a model to compare fossil energy use in organic and conventional farming. In general, there is lower energy consumption in organic farming but also lower yields. Self energy production is the natural next step in development of organic farming.

BioConcens project (its full name: Biomass and bioenergy production in organic agriculture – consequences for soil fertility, environment, spread of animal parasites and socio-economy), focuses on bioenergy production from local biological resources, and at the same time analyzing the effect of bioenergy production on soil fertility, greenhouse gas emissions, survival of parasites and weed seeds, and socio-economy.

“This interdisciplinary project aims at developing new methods and processes for the co-production of bioethanol, biogas and animal feed based on resources from organic agriculture and associated food processing and suggests the outline of a medium-sized plant for co-production of biogas, bioethanol, and animal feed. The project also designs and tests a new cropping system for biomass production to be used for bioenergy, while at the same time safeguarding soil quality. The project analyzes the effects of remains from bioenergy production on soil fertility, greenhouse gas emissions, survival of parasites and weed seeds in the manure as affected by bioenergy production. Corporate and socio-economic analysis of the co-production of biogas and bioethanol at different scales is carried out” (<http://www.bioconcens.elr.dk>).

At the time of writing, this project is still on-going and the final results and conclusion are not yet available. Dalgaard et al. (2009) discussed synergies between the expansion of biogas production and organic farming, concluding that a 150% increase in organic farming in combination with bioenergy crop production is possible and would contribute to the vision of independency from fossil fuel in Denmark. Carter et al. (2009) measured and reported the amount of N_2O and CH_4 emissions when the residues from bioenergy production are recycled as organic fertilizer for energy maize cultivation. The effect on soil fertility when waste streams from bioethanol and biogas processes are recycled on the fields as fertilizers was studied in (Johansen et al., 2009) demonstrating almost no difference on soil quality between degassed and fresh manure. The strip intercropping (Haugaard-Nielsen et al., 2007) method was applied and studied in test fields for energy crop production to enhance soil fertility (Haugaard-Nielsen et al., 2009). Pugesgaard et al. (2010) evaluated the impact on the environment when biogas is produced at the organic farm. The initial results focusing on energy balance, nitrogen losses and greenhouse gases emission on the organic farm with integrated bioenergy production were presented in (Pugesgaard et al., 2008). More details and the full list of publications can be read on the project’s website (<http://www.bioconcens.elr.dk>).

The presented work in this thesis is focused on the “technological” part of the project. In the depicted concept, the bioenergy is produced from animal manure originating from dairy farms, a by-product from cheese production (agro/food industry) – whey permeate, and energy crops cultivated at the farm. The effluents from the bioenergy plant could serve either as natural fertilizer or protein feed, depending on the applied

technology. The bioenergy plant could be designed either for a single or several combined organic farms. Depending on the scale, the generated energy could supply only an organic farm or serve broader community. The whole scheme and the concept of the project are presented on the Figure 1.

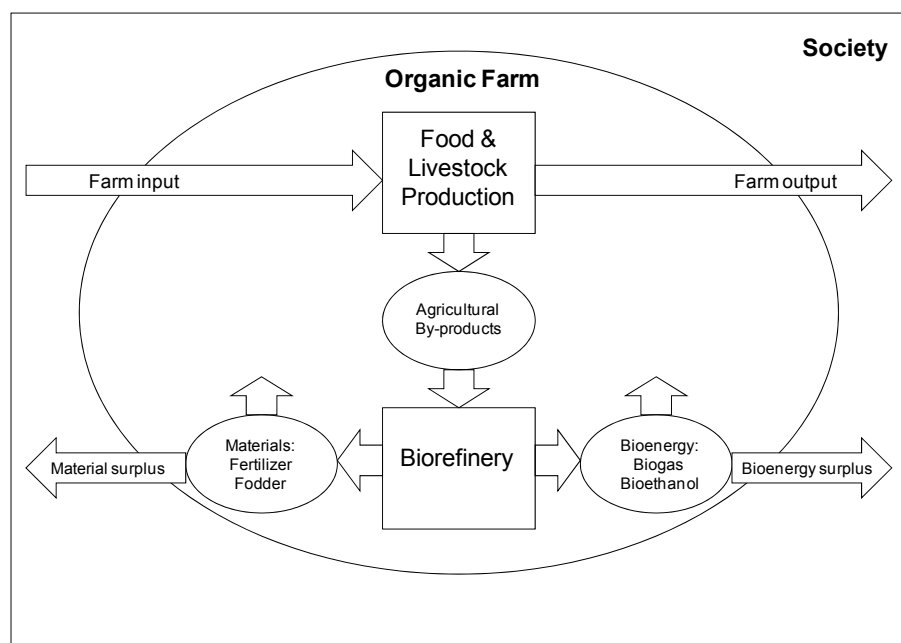


Figure 1. On-farm bioenergy production in BioConcens project

In our investigation, we used a model organic farm which is based on a statistical data on organic farms in Denmark in 2006. The organic farm consisted of: whole crop production (maize, grass/clover – silage in rotation, permanent grass), cash crops, grain production (spring barely, spring wheat, oats, winter wheat, winter rye, and triticale) and set aside and fallow land. Its distribution is presented in Figure 2. Detailed description can be found in (Pugesgaard et al., 2010). This baseline was used during the further evaluation of producing bioenergy at the organic farm.

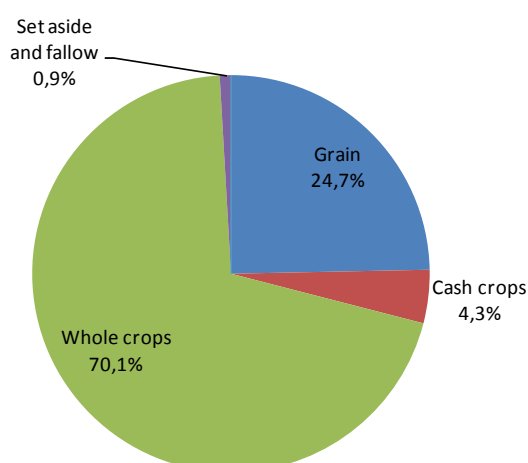


Figure 2. The crop distribution for the baseline in an exemplary organic farm in Denmark (based on Pugesgaard et al., 2010)

3. Biomass and bioenergy

Nowadays, world energy supply is dominated by fossil fuels (80% world's primary energy mix), biomass usage accounts for (11% world's primary energy mix), however part of it is utilized for simple cooking with very low efficiency. Modern bioenergy from biomass–commercial energy production for industrial purposes, power generation and transportation – is at the level of 7% (WEO, 2008). Figure 3 presents current and future energy trends and share of biomass in world energy supply.

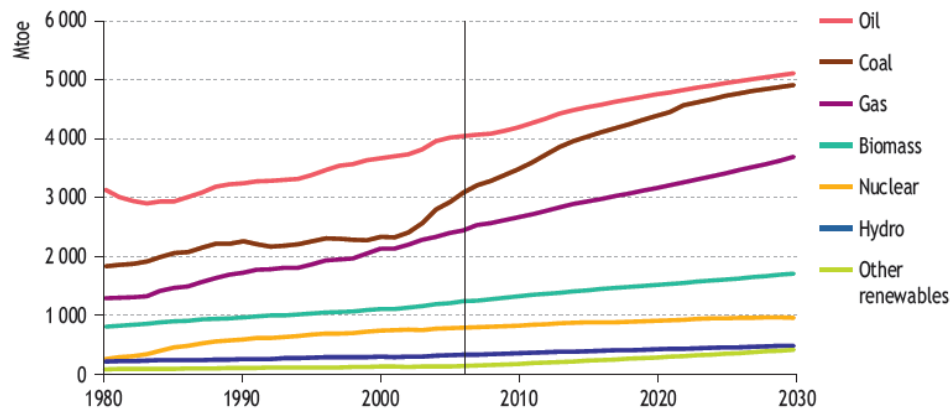


Figure 3. World primary energy demand by fuel (reference scenario) (WEO, 2008)

According to the International Energy Agency the world energy demand will expand by 45% from 2006 till 2030 and it will reach 17 Mtoe. It is estimated that fossil fuels will continue to dominate global energy use; the total energy demand for Europe in 2030 is estimated for almost 2000 Mtoe.

The largest increase in renewable energy use, in the coming years, will take place in the EU countries, driven by strong governmental support - the European Union strategy to lower the CO₂ emission, strengthen the security of energy supply and create diverse, efficient and sustainable energy mix. European Commission suggested the share of renewable energy should be in the range of 20% by 2020 and 50% and more by 2040-2050 (EC, 2006).

Diversifying energy sources would increase the security of supply. Building the new energy structure, based on different renewable resources should be the main target, where biomass, wind, solar, and hydro become an integrated part of the overall energy strategies, with an important sustainable role for bioenergy and biorefineries to play. (Biofuels Progress Report, Holm-Nielsen et al., 2007)

Development and implementation of improved growing systems for the purpose of biomass production for biorefinery utilisation will get more and more important, due to increasing demands for biofuels and a variety of biorefinery products. The commitment of making this kind of shift in using sustainable resources at much larger scale is growing already and will grow in the coming decades. Such a tendency is common all over the world: in rapid developing countries, like in China or India as well as in developed

regions. On the other hand, in many poorly developed countries in Africa and Asia, the biomass as an energy source is the only way to provide the heat and electricity to the society. The question is how will nature be influenced, and will the environment be harmed by increasing biomass production for the worldwide energy sectors. The greatest challenge will be to make the paradigm shift from fossil fuels to renewable resources in a sustainable manner (Holm-Nielsen et al., 2007). Johansson et al., (2010) raised the question whether agriculture does have a capacity to provide us with both food and fuel. Both biogas and bioethanol can be produced from residues but how much residues can be removed from fields without a risk of soil degradation. Johansson et al. (2010) claimed that with present rate of population increase, the challenge will be to assure food security. However, it was concluded that the calculated global potential of biogas could supply up to one-fourth of present motor fossil fuels. At present the global food production is sufficient to feed the world population, famine is rather a matter of its distribution, however there is a concern that this situation might change with growing world's population. There are many advantages from utilizing bioenergy, but there is also a great challenge, concern and responsibility, that cultivation of energy crops might reduce land availability for feed and food production (Holm-Nielsen et al., 2007).

It is estimated that around three-fourths of the biomass which is used for production of food, feed, industrial round wood and traditional wood fuel is lost at some point in processing, harvesting and transport (Smeets et al., 2007). Part of recovered biomass could be easily applied for bioenergy. Moreover, higher efficiency of production of food/feed, industrial round wood and traditional wood fuel means that there would be more available biomass for modern bioenergy production (Holm-Nielsen et al., 2007).

Crop residues might be significant source for bioenergy. However, removal of large quantities of residues from cropland has to be consistent with research-based guidelines in order to do it in a sustainable manner. In some cases removing any residues can cause loss of soil carbon, whereas on other soils some level of removal can be sustainable and even beneficial. Residue removal should not result in increased artificial fertilizer application, in this case the environmental and economy effects can be negative (Perlack et al., 2005).

3.1. Raw materials

Plant biomass can be considered as one of the most sustainable resource for organic fuels, chemicals, and materials. Growing plants consume CO₂ - therefore biomass-based products can be included in photosynthesis carbon cycle reaching almost CO₂-neutral lifecycle (Figure 4). Moreover, the biological processes are mostly carried out in aqueous environment and the effluents are non-toxic to the environment and easy to discharge. In some cases, the effluents can be even valuable by-products (Demirbas, 2006).

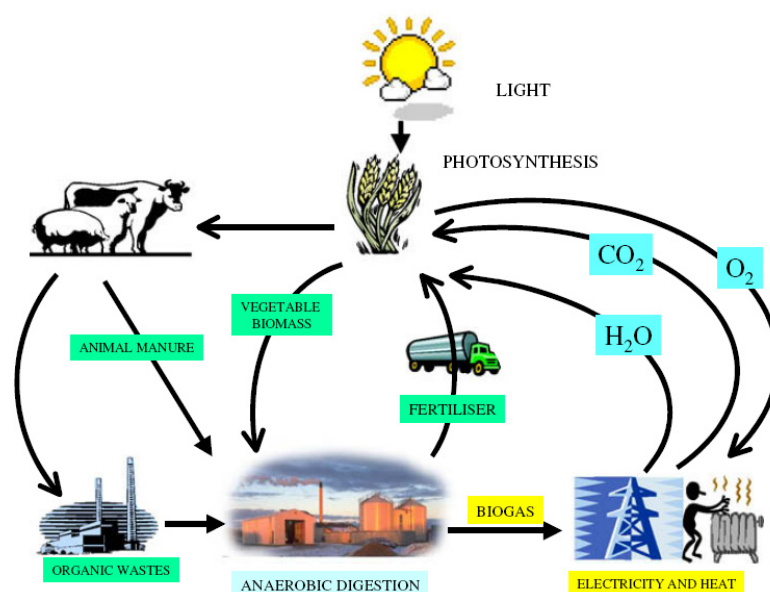


Figure 4. Schematic representation of the sustainable cycle of bioenergy (anaerobic co-digestion of animal manure and energy crops) (Al Seadi, 2002; Holm-Nielsen et al., 2009)

Biomass resources occur in variety of ways, such as woody and herbaceous species, wood wastes, bagasse, agriculture and industrial residues, waste paper, municipal solid wastes, sawdust, biosolids, grass, waste from food processing, animal wastes, aquatic plants and algae, and so on (Demirbas, 2008). Different global energy scenarios indicate that biomass could supply up to 30% of the energy needs by 2100 (Hamelinck and Faaij, 2006).

Through photosynthesis process, plants convert carbon dioxide and water to metabolite chemicals. Primary metabolites are carbohydrates (simple sugars, cellulose, hemicelluloses, starch etc.) and lignin – all together called lignocelluloses. Cellulose and hemicelluloses are two principle polymers and from those ethanol can be produced. Cellulose is β (1,4) linked polymer of glucose, it has high degree of polymerization and cristallinity. Hemicellulose is highly branched polymer built up from hexoses and pentoses (Carpita and Gibeaut, 1993). Lignin, the third component, is a complex hydrophobic cross-linked aromatic polymer which serves as “glue” for lignocellulosic structure. Secondary metabolites are mainly gums, resins, rubber, waxes terpenes, tepenoids, steroids, plant acids etc (Clark, 2007; Naik et al., 2010).

Energy carries from biomass can be produced in a variety of ways including liquid fuels such as ethanol, methanol, biodiesel, Fisher-Tropsch diesel, and gaseous fuels such as hydrogen and methane. There are also several ways to convert biomass into fuel, mainly thermo-chemical (combustion, gasification, pyrolysis, liquefaction) and biochemical (anaerobic digestion, fermentation).

Choice of raw material is crucial. Lignocellulosic biomass is the most abundant organic material on Earth and that is why is very interesting for bioenergy production (Wyman, 1996). In **Papers I and II** composition of several raw materials available at the organic farm which are suitable for biogas or bioethanol production is shown. Compositional

analysis is necessary to estimate overall efficiency of the process whether it is biogas or bioethanol: for the first one overall amount of organic matter (VS) is crucial. Very often it is a basic characterisation of inoculums and substrates i.e. (Lehtomäki et al., 2007). On the other hand, for ethanol fermentation the sugar concentration of lignocellulosic materials is the most important (Foyle et al., 2007). Example of such a characterisation is presented in (Petersson et al., 2007; Xu et al., 2010). Total and volatile solids of raw materials are shown on Figure 5; composition of investigated energy crops is summed up on the Figure 6.

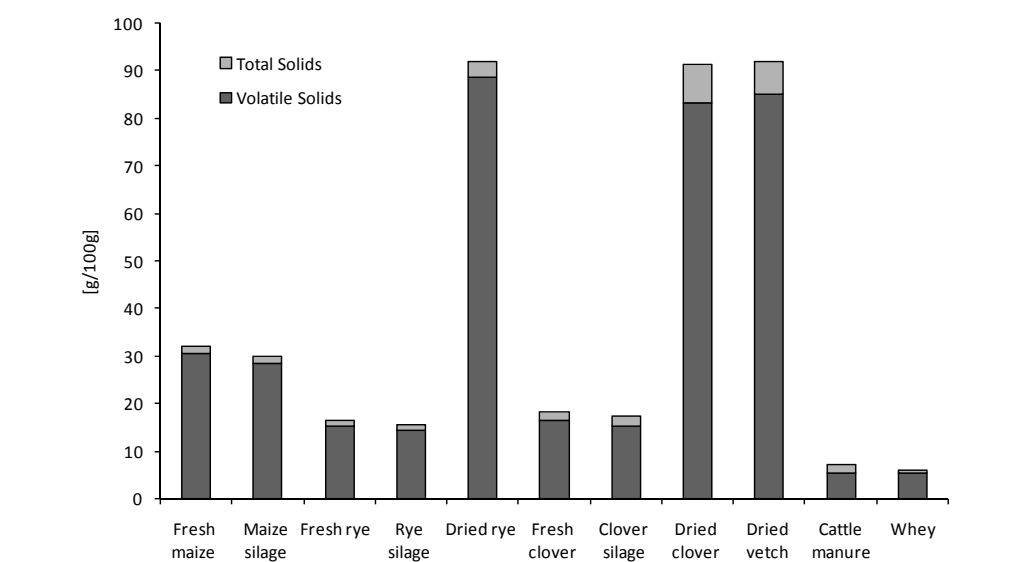


Figure 5. Total and volatile solids of the raw materials

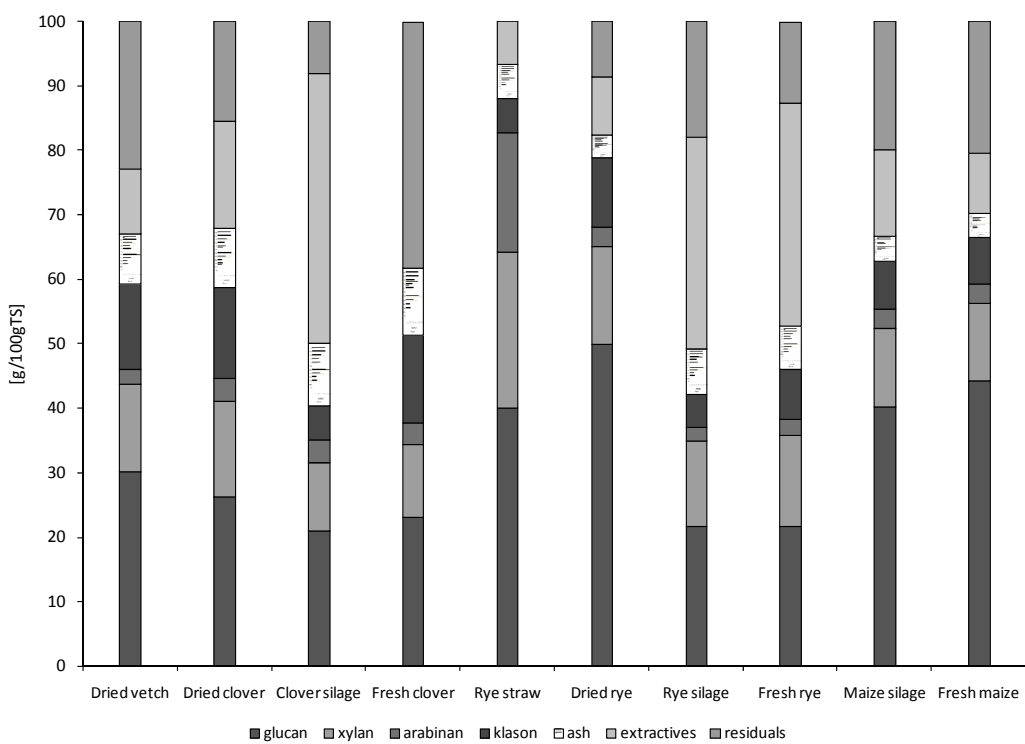


Figure 6. Composition of investigated organic energy crops

Each of the characterized biomass differs in amount of water, primary and secondary metabolites depending on the time of harvest, method of storage as well as type of plant. The dry part of the biomass is referred as TS (total solids) or DM (dry matter). In fresh green biomass samples: waxes, tars, colorants can be found – those are referred as extractives. The main organic compounds in biomass can be classified as cellulose, hemicelluloses and lignin. Cellulose is built from glucose monomers (C6) whereas hemicelluloses from xylose (C5) sugars. Additionally in fresh green biomass samples or in silage samples sugars like fructose can be found.

3.2. Ethanol

One of the best substitutions for fossil fuels could be bioethanol (Mandil, 2004), in 2009 74 billion liters of bioethanol were produced worldwide: 90% of comes both from US (from starch) and Brazil (sugar cane) (RFA, 2010). Ethanol as fuel has great property such as high octane number however the energy density is lower compared to gasoline. Different mixtures of ethanol and gasoline are available, the most popular in Brazil is E85 (contains 85% of ethanol) but it requires so called flex-fuel car. More common blends elsewhere E10 or E5 are suitable for unmodified cars. Detailed properties of ethanol containing fuels can be found in (Hsieh et al., 2002). Bioethanol also fits to the existing infrastructure and it can easily replace gasoline, which is very strong advantage of that fuel.

3.2.1. Ethanol potential

Ethanol produced via microbial fermentation can be produced from fermentable sugars: C6-glucose derived from starch or cellulose (from lignocellulosic biomass) or from C5-xylose derived from hemicelluloses. So called, 1st generation ethanol based on starch is developed and mature technology, whereas 2nd generation ethanol (produced from lignocellulosic materials) is during the development (Larsen et al., 2008).

To estimate overall efficiency of the process of ethanol production from lignocellulosic materials, composition of substrate is necessary, mainly sugars concentration (Foyle et al., 2007). In **Paper II**, bioethanol potential of four different crops (maize, rye, clover grass and vetch) available on the organic farm was estimated. The study considered crops in diverse conditions (fresh, ensiled or dried) depending on type of the crop and common practice of storing it. Theoretical yields based on C6 and/or C5 sugar content were summarized. Results were presented in volume of ethanol produced per mass of raw material as well as energy content of produced fuel per area necessary to cultivate it. On the Figure 7, the theoretical ethanol potential, based on C5 and C6 sugars is shown. The numbers represent maximum ethanol which could be achieved through fermentation process, however after applied pretreatment method; the amount of produced ethanol would be lower. Xu et al. (2010) investigated hydrothermal method on maize silage, achieving from 55% to 77% of the theoretical one, in (Oleskowicz-Popiel et al. 2008) after wet-oxidation method, 82% of theoretical ethanol was produced from maize silage. Petersson et al. (2007) studied, among other materials, winter rye resulting in yield of 66% of the theoretical after wet-oxidation pretreatment method whereas Martin et al. (2008) produced around 87% of the theoretical ethanol from wet-oxidized

clover-ryegrass mixtures. Other authors showed results ranging from 60-90% of theoretical ethanol (Linde et al., 2008; Wyman et al., 2009; Carrasco et al., 2010). Obtained yields depend not only on the type of raw materials but also pretreatment method, concentration of enzymes and microorganisms, types of organisms and overall process conditions. Due to the fact that different lignocellulosic materials have different physico-chemical characteristics, almost each type of biomass has special optimal pretreatment conditions; the only way to compare full ethanol potential of several raw materials is through compositional sugar analysis.

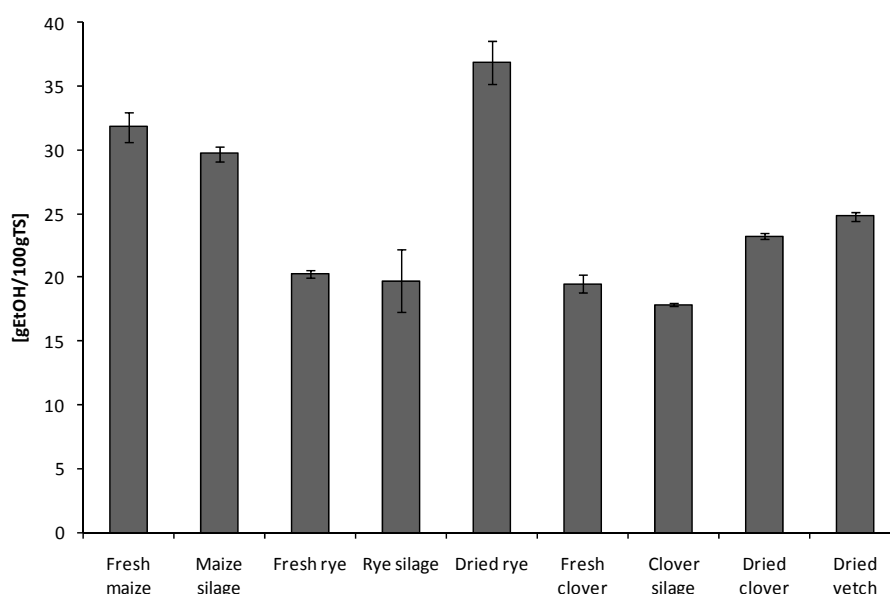


Figure 7. Theoretical ethanol potential in investigated raw materials (**Paper II**)

3.2.2. Pretreatment

Pretreatment refers to “disruption of the naturally resistant carbohydrate-lignin shield that limits the accessibility of enzymes to cellulose and hemicelluloses” (Yang and Wyman, 2008). One of the main technological challenges in lignocellulosic ethanol is to develop optimal pretreatment process (Chandra et al., 2007; Yang and Wyman, 2008). Over the years, several methods have been developed and are reviewed in (Wyman, 1996; Olsson et al., 2005; Alvira et al., 2010), to mention the most successful:

- Dilute acid pretreatment – dilute sulfuric acid pretreatment (Torget et al., 1991), besides achieving very high yields it has several disadvantages such as very corrosive environment and reaction degradation products such as furfural and acetic acid, which are strong inhibitors for microorganisms;
- Ammonia explosion – (Chou, 1986) the main advantage is low process temperature and low inhibitors formation, however it has high cost of ammonia and it is not suitable for woody substrates;
- Steam explosion – (Saddler et al., 1983) pretreatment is performed without presence of chemicals but with moisture. Hydrolysis is catalyzed by organic acids liberated from the biomass;

- Hydrothermal treatment – (Bonn et al., 1983) this technique involves cooking the biomass in water in high temperature.

Several other techniques have been investigating (Bjerre et al., 1996; Rossgard et al., 2007; Yang and Wyman, 2007, Galbe and Zacchi, 2007), where some of them were successfully applied in pilot scale for bioethanol production i.e. (Thomsen et al., 2006).

There is growing interest for a small farm-scale production renewable energy (Ahlgren et al., 2008). Especially organic farmers are interested and forced in improving their sustainability by using “green” energy and at the same time to make their farms self sufficient in terms of energy supply. As a result of this, there is a need for new and low-tech processes for bioethanol production with pretreatment techniques that will not interfere with organic farming principles and requirements (IFOAM) and at the same time are efficient.

Silage pretreatment - wet storage method can be one solution; it can be used to both preserve and pretreat biomass feedstock (Ren et al., 2006). Originally, ensiling is method for forage storing and preserving (Charmley 2001), which for long time has been used all over the world (Weinberg and Ashbell, 2003). The purpose of silage making is to store and preserve crops with minimum loss of nutrients i.e. feed value. Ensiled material, often referred to as silage, consists of the whole harvested plant (stem, leaves, and grain) and grasses which is used for animal feed. In a correct ensiling, lactic acid bacteria dominate the fermentation process; the low pH caused by fermentation of part of free sugars preserve the feedstock from further degradation by inhibiting fungus microbes, in that way effectively minimizing the degradation of sugars in a crop (Thompson et al., 2005). In conventional silage process without additives, half of the hemicelluloses content can be degraded but less than 5% of cellulose (Ren et al., 2006). (Yahaya et al., 2001) investigated polysaccharide degradation in orchardgrass and lucerne during ensiling, noticing 17.2-19.8% hemicellulose degradation and only 0.5-3-3% of cellulose. Similar conclusions, that hemicellulose is easier hydrolyzed than cellulose during that process, were found by (Kawamura et al., 2001).

Silage crops have been already widely used for biogas production (Zubr, 1986; Amon et al., 2007; Vervaeren et al., 2010) they were also used in ethanol trials but after wet-oxidation, hydro-thermal or steam pretreatment (Thomsen et al., 2008; Xu et al., 2010; Sipos et al., 2010). Investigation concerning ensiling as a stand-alone method for wet storage and pretreatment process for production of 2nd generation ethanol was presented in **Papers III and IV**.

Fresh maize, rye and clover grass were ensiled and the influence of the silage process was described and discussed in **Paper III**. The ensiling method in laboratory conditions was described in Materials and Methods in **Paper III**. Ensiled samples were compared to fresh crops. Enzymatic convertibility tests and fermentation trials were carried out on all investigated biomass. Very promising results were achieved and silage process has been proved to be efficient wet-storage method which additionally could serve as sterilization and mild pretreatment method for second generation ethanol.

Paper IV goes with one step further, dry lignocellulosic by-product – corn stover – is moistened and stored in a silage form (described in Materials and Methods section, **Paper IV**). Noticeable positive influence of the ensiling proved once again that it can be a successful storage method for lignocellulosic materials. Ren et al., (2006) investigated ensiling of corn stover as a long term feedstock preservation method concluding that it can guarantee stable 6 month biomass preservation.

Previous authors (Chen et al., 2007; Digman et al., 2010) already indicated that ensiling could be applicable in bioethanol industry. Chen et al. (2007) wrote that the ensiling significantly increased the conversion of cellulose and hemicelluloses to sugars during subsequent enzymatic hydrolysis. It was concluded that it is not as efficient as chemical pretreatment but it is low-cost and energy conserving technique. Digman et al. (2010) evaluated ensiling with and without chemical addition as a wet storage for switch grass and reed canary grass prior to conversion into ethanol. It was found out that addition of sulfuric acid was more effective compared to lime addition. Both investigations indicated that ensiling is a very promising method for wet storage of lignocellulosic biomass and it increases overall ethanol yield. It is in accordance with results presented in **Paper III** and **IV**. Deeper understanding of the process and its optimization from feed preservation method towards lignocellulosic pretreatment for second generation bioethanol would be necessary.

3.2.3 Ethanol fermentation

The production of ethanol consists of several different steps (Aden et al., 2002). After choice of raw material, the next step is hydrolysis, which purpose is to split sugars from cellulose and hemicelluloses into monomeric sugars. It includes pretreatment (which is described above in point 3.2.2) and enzymatic hydrolysis. During enzymatic hydrolysis the polymer of cellulose is reduced to simple sugars. Typically cellulase enzymes are classified as follows (Petersson, 2005): endo- β -glucanases (cleave the polymer randomly), exo- β -glucanases (cleave off units of cellobiose), exo- β -glucosidase (cleave off glucose) and β -glucosidases (cut cellobiose into two units of glucose). The cellulase enzymes are rather costly and the enzyme loading should be minimized, but not to increase the time needed to complete hydrolysis (Olsson et al., 2005). In most cases cellulase enzyme are produced by *Trichoderma reesei* and *Aspergillus niger* (Hendy et al., 1984; Lo et al., 2010).

Recent review articles in that field describe current advances, opportunities and obstacles in successful enzymatic hydrolysis process (Meyer et al., 2009; Alvira et al., 2010; Talebnia et al., 2010). Several factors influence the results from enzymatic hydrolysis test (Alvira et al., 2010): cellulose crystallinity, degree of polymerization, available surface area, lignin barrier, hemicelluloses content, feedstock particle size, porosity and cell wall thickness. Enzymatic convertibility test can serve as first indicator on digestibility of raw material to produce biofuel, it can also give an idea about pretreatment severity necessary to open lignocellulosic structure.

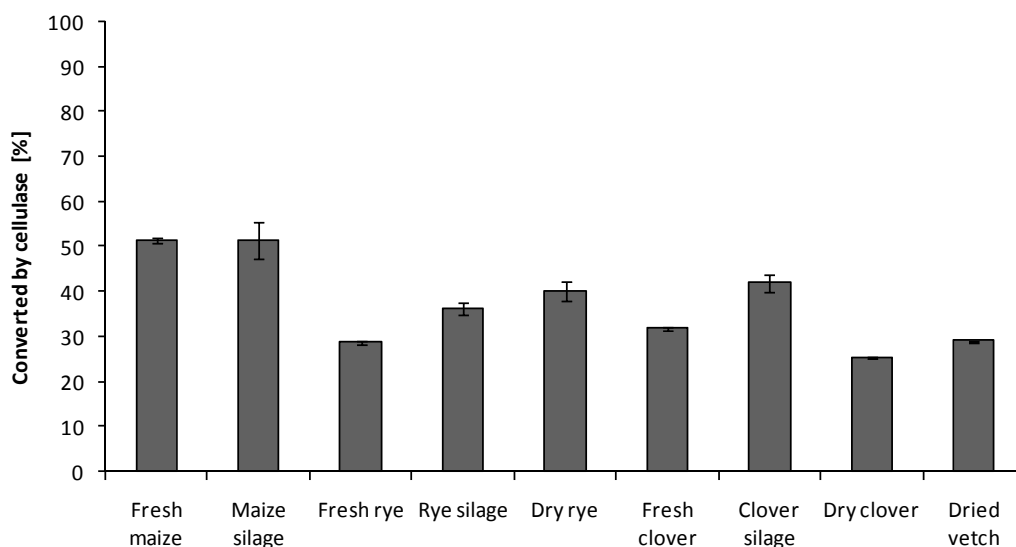


Figure 8. Results from enzymatic convertibility test of raw materials presented in Paper III and IV

Cellulase dosage of 10-30 FPU/ g cellulose is often used in laboratory trials because it gives high glucose yields in reasonable short time (Talebnia et al., 2010). The loading depends on substrate, pretreatment method; in laboratory experiments often exceed the loading applied in pilot or demonstration scale. Results from enzymatic convertibility tests presented in **Papers III** and **IV** and summarized on the Figure 8 aimed to characterized different raw materials for their digestibility for ethanol production.

In the fermentation process the monomeric sugars revealed during enzymatic hydrolysis, are converted into ethanol by microorganisms. Talebnia et al. (2010) reviewed different microorganisms used or studied to produce ethanol. The most common and at the same best performing are typical Baker's yeast – *Saccharomyces cerevisiae*. Those are also the organisms used in some of the test presented in **Paper III**. The main advantage of *S.cerevisiae* is its robustness, it is well suited for diverse agricultural raw materials where possible inhibitors can occur (Klinke et al., 2003), it also gives a high ethanol yield and high ethanol productivity. The main disadvantage would be disability of fermenting C5 sugars (arabionose and xylose), which also occur in lignocellulosic feedstock. Second strain applied in the experiments was thermo-tolerant yeast *Kluyveromyces marxianus* (**Paper III, IV** and **V**). The advantage was higher processing temperature (40°C), which were closer to optimum for cellulose hydrolysis (50°C) (Kadar et al., 2004); consequently higher rates of hydrolysis were expected.

In order to evaluate ensiling method as a wet-storage or pretreatment, batch fermentation trials were performed. In **Paper III**, two kinds of yeast were applied. Higher ethanol production was observed for the thermophilic yeast, which is explained by the higher process temperature (40°C compared to 32°C for Baker's yeast) being close to the optimal hydrolysis temperature for cellulase enzymes (50°C) - consequently more glucose was available to be converted into ethanol.

Part of the concept for the decentralized biorefinery at the organic farm (within BioConcens project) is to produce ethanol from whey. Whey, which is by-product from cheese industry, contains lactose – a disaccharide consisting of glucose and galactose. It cannot be fermented by *Saccharomyces cerevisiae*, which is commonly used in alcohol fermentation, because this strain of yeast lacks β -galactosidase activity. *K.marxianus* is capable of fermenting lactose to ethanol directly. *K.marxianus* was studied extensively and was the best choice for this raw material (Wang et al., 1987). Laboratory trials on that subject are described in **Paper V**. Main conclusion were that even without pasteurization or freezing of whey, *K.marxianus* successfully competed with lactic acid bacteria, producing high ethanol yield (0.50 g ethanol/ g lactose). Additionally, during continuous trials high ethanol productivity was achieved (2.5-4.5 g/L/h).

3.3. Biogas

3.3.1. Process principles

Anaerobic digestion is a biological process where most organic matter (carbohydrates, lipids, proteins) except for lignin components, in the absence of oxygen, is degraded into methane and carbon dioxide. The process consists of series of reactions and it is a natural process which takes places in several anaerobic environments. In anaerobic digestion processes can be divided into (Gujer and Zehnder, 1983; Angelidaki et al., 2002), the schematic view is shown on Figure 9:

- Hydrolysis - the fermentative bacteria hydrolyze biopolymers such as proteins, carbohydrates and lipids into oligo- and monomers by extracellular enzymes. The proteolytic bacteria produces proteases to hydrolyze proteins, the cellulytic and xylanolytic bacteria produces cellulases and xylanases to degrade carbohydrates and lipolytic bacteria produces lipases to hydrolyze lipids.
- Fermentation - during this process organic material will be transformed to methanogenic substrates (hydrogen, carbon dioxide and acetate) and lower fatty acids and alcohols. The main process is acetogenesis, where volatile fatty acids (VFA) and alcohols produced during fermentation step are oxidized to acetate – this reaction is catalyzed by acetogenic bacteria.
- Methane formation – the methanogenic bacteria are divided into two groups: the aceticlastic methane bacteria, which degrade acetate; and the hydrogen consuming methanogens. Methanogenesis is an energy producing process and it is regarded as the motive force for anaerobic digestion.

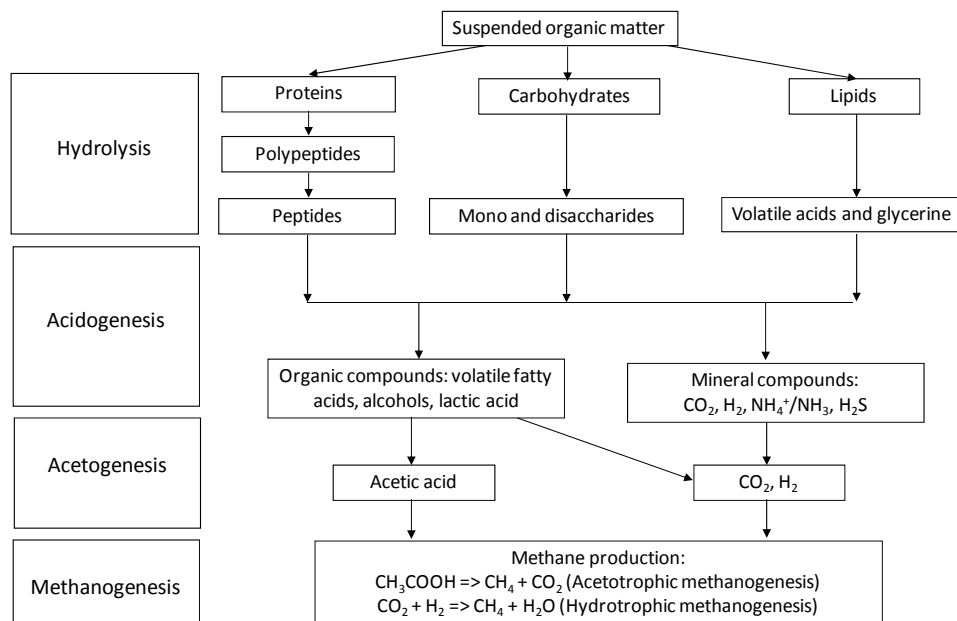


Figure 9. Schematic view of anaerobic digestion process (adapted from (Gujer and Zehnder, 1983; Angelidaki et al., 2002))

Anaerobic digestion, not only provides renewable energy source (biomethane) but it also deliver highly efficient natural fertilizer (Angelidaki et al., 2003). Möller and Stinner (2009) investigated effects of different manuring systems. They concluded that biogas digestion of field residues resulted in a win-win situation. Besides additional energy, there is a lower nitrate leaching and lower nitrous oxide emission, the disadvantage is higher ammonia volatilization compared to undigested manures. Anaerobic treatment also minimizes the survival of pathogens which is important in applying it as fertilizer.

Such benefits are very suitable for organic farmers, which are very concern about soil fertility and nutrients recycling. The greenhouse gas emission reduction and sustainable development of energy supply makes this technology one of the most promising for on-farm application (Svensson et al., 2005; 2006). Biogas as renewable energy source will play vital role in the future, it can replace fossil fuels for heat and electricity generation as well as vehicle fuel. If upgraded, it can be injected into natural gas grid, moreover biomethane can be a feedstock for producing other chemicals and materials (Holm-Nielsen et al., 2009; Weiland, 2010).

3.3.2. Biogas potential

Substrates type and its composition directly influence the biogas yield. The input to the process can be measured in chemical oxygen demand (COD) or total/volatile solids (TS/VS) values. It is crucial to determine the degradable and inert fraction of the feedstock. Animal manure, which in most cases is principle compound of feed, has low methane yield per COD or VS compared to other applied raw materials (Møller et al., 2004). Lignin is one of the non-degradable compounds. On the other hand, many industrial organic wastes contain significant amount of easily degradable compounds. In

Paper I and II biogas potentials of diverse crops available at the organic farm were presented (summarized on Figure 10).

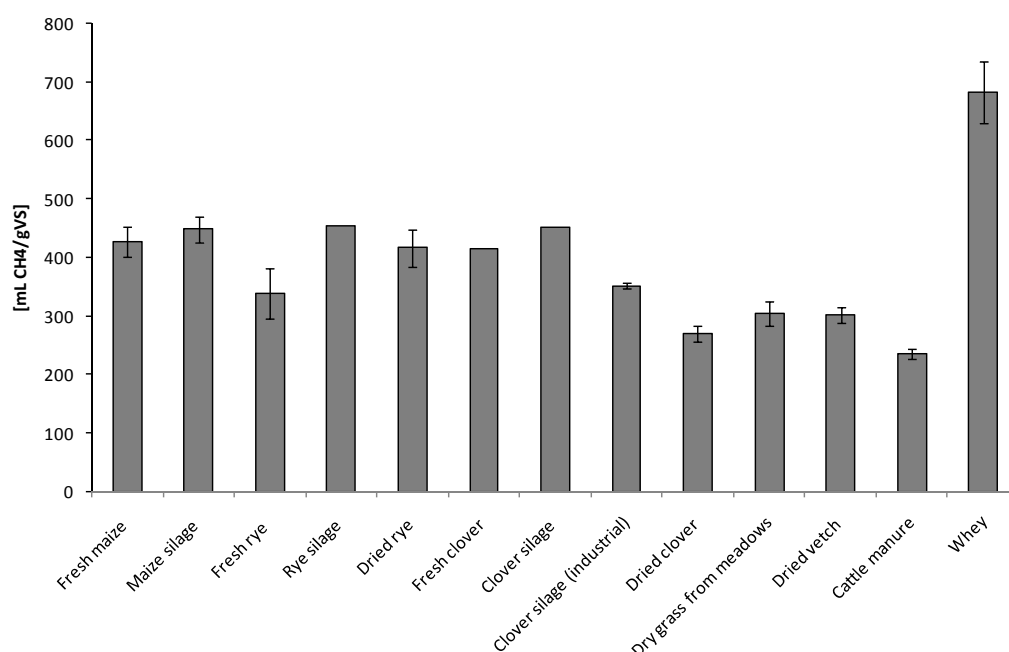


Figure 10. Methane potential of different raw materials available at the organic farm

Estimation of methane potentials differs from estimation of ethanol potentials (described previously). During anaerobic digestion most of the organic compounds are degraded. One way would be to completely characterize raw materials and calculate methane potential based on proteins, carbohydrates and lipids concentrations. This is however complicated and expensive. Rough estimation can be based on COD or VS content but it does not give precise results. The most common procedure is practical methane yield determined during batch laboratory experiments. Determining methane potential created several doubts how the test should be performed to obtain reliable and comparable results. In (Angelidaki and Sanders, 2004) attempt to systematize and unify the methane potentials was taken, where review of different methods is presented. Hansen et al. (2004) identified optimal process conditions for determining methane potential from organic solid wastes. It included ratio inoculum to sample, number of replicates, the origin of inoculum, process time and method for measurement of produced methane. Angelidaki et al. (2009) suggested common method for biomethane potential of solid organic wastes and energy crops, it was recommended to:

- Characterize substrate for total solids, volatile solids, chemical oxygen demand, nitrogen and phosphorus content, additionally content of lignin, cellulose and hemicelluloses could be determined;
- “Fresh” and “degassed” inoculum pre-incubated in the same temperature as process temperature, appropriate volume of inoculum according to its activity;
- Necessary nutrients/micronutrients/vitamins should be supplied unless they are present in inoculum or substrate;
- Blank assay should be always carried out;

- The number of replicates should be at least three for each dilution;
- Some mixing should be applied (e.g. turn up down once a day);
- For new substrates with unknown degradation characteristics, a number of different dilutions of the substrate (with water) are required;

Detailed description of assay experimental set up, guidelines and advices for can be found in (Angelidaki et al., 2009).

3.3.3. Continuous trials

Continuous trials with cattle manure and maize silage are presented in **Paper II**. In co-digestion process manure provides buffering capacity and nutrients while an energy crop with high carbon content balances carbon to nitrogen ratio of the feedstock. Such a combination has been proved to result in higher methane yields (i.e. Parawira et al., 2008). Our trials showed that methane yield in co-digestion of cattle manure and maize silage resulted in 267 mL CH₄/gVS_{added}. With 33% of maize silage in the feedstock, 36% increase of methane production was read. Methane yield originated from maize silage varied between 304 and 384 mL CH₄/gVS. Lehtomäki et al. (2007) investigated co-digestion of cow manure with sugar beet tops, grass silage and oat straw achieving methane yield of 229, 268 or 213 mL CH₄/gVS_{added}, respectively, where feed contained 30% of crop. Further increase of crop in the feedstock decreased methane yield. Comino et al. (2010) after mechanical pretreatment (chopped to a size of 2mm) of silage crop mix, increased till 70% VS crop portion in the feedstock. It resulted in 109% higher specific methane yield compared to start-up phase (only with manure). Further increase of crop percentage in the feed decreased methane production. Lindorfer et al. (2008), on the other hand, claimed that up to 96.5% VS of energy crop ratio is possible in a feedstock without any decrease, however longer adaptation time of the microbial population to the feedstock is required. Apart from high methane yield, digestion of only energy crops might have disadvantages, Lebuhr et al. (2008) studied mono-digestion of maize silage, it was found out that long term trace elements (cobalt, molybdenum, selenium) have to be supplemented.

3.4. Ethanol and biogas co-production

Process integration can lead to more intensive and cost-effective on-farm energy production. "Integration opportunities may provide the ways for a qualitative and quantitative improvement of the process so that not only techno-economical, but also environmental criteria can be met" (Cardona and Sanchez, 2007). One of the concepts for process integration within BioConcens project was to co-produce ethanol and biogas from germinated grains, whey and optionally clover grass silage.

Malting, normally used in brewing of beer, develops enzymes that are required to hydrolyse the complex starch in grain into simple fermentable sugars. Natural enzymes from cereals were used for hydrolysis of starch to glucose in accordance with technology in brewing technology. Enzyme production during germination was extensively studied on barley (Briggs et al., 1981). Biorefinery concept where bioethanol is produced from

germinated grains and whey and rich in protein effluent is as animal feed and remaining process water is treated in upflow bioreactor to produce biogas is presented on the Figure 11.

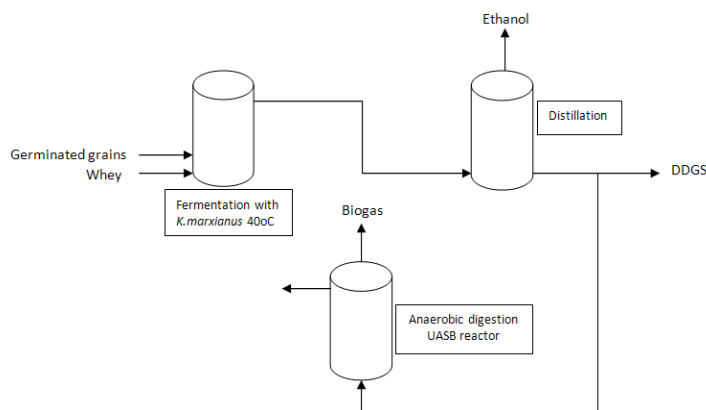


Figure 11. Concept for co-production of ethanol and biogas from germinated grains and whey

The effluent was separated into two streams: the solid part to be used as rich in protein animal feed, the liquid part should be further processed in UASB reactor (up-flow anaerobic sludge blanket reactor) to produce biogas from remaining organic compounds. Biogas production from whey was studied broadly (Hwang and Hansen, 1992; Kalyuzhnyi et al., 1997; Kato et al., 1997). Ergüder et al. (2001) concluded that undiluted cheese whey could be treated anaerobically at relatively short retention time (2.06-4.95 days) without any significant stability problems. Alternatively whey could be treated by co-digestion with manure in CSTR reactor. Gelegenis et al. (2007) achieved stable biogas production with whey fraction until 50%, above that the reactor turned to be unstable. From initial experiments following results were obtained:

- From mixture of: 14g (73%TS) of germinated grains and 86g (6.5%TS) of whey: 2.9g EtOH and 4988 mL methane was achieved
- From mixture of: 7g (73%TS) of germinated grains, 73g (6.5%TS) of whey and 20g (18%TS) clover grass silage: 2.2g EtOH and 4641 mL methane was achieved

Moreover, the biogas potential of each specific compound of effluent was measured and it is shown in Table 1.

Table 1. Methane potential of investigated feedstock

Feedstock	[mLCH ₄ /gTS]
Whey	~700
Fresh clover silage	~440
Effluent clover silage	~400
Grains	~600

Further experiments and development of this biorefinery concept continues.

4. Biorefinery modeling

4.1. Principles

Modeling and simulation of chemical and bioprocesses helps to identify possible improvements as well as to identify potential difficulties. During the development of the process, to some extent simulation can act as a substitute for the experimental part (Heinzle et al., 2006). The principle steps in the process modeling are presented in the Figure 12.

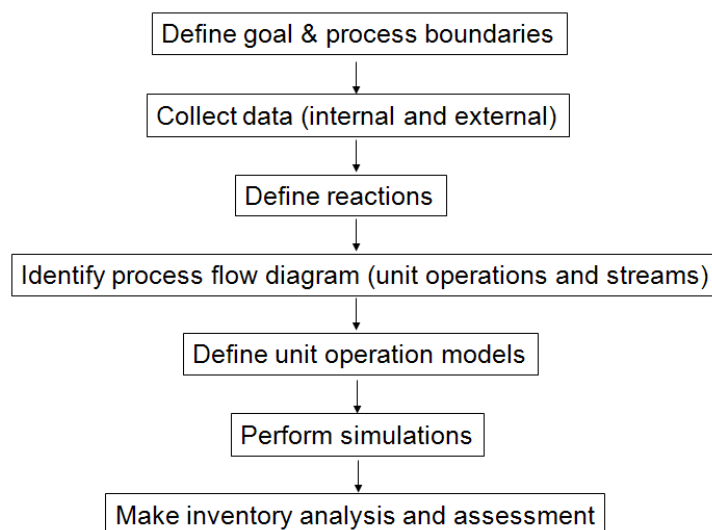


Figure 12. Basics steps in process modeling (adapted from Heinzle et al., 2006)

The definition of the goal, the model boundaries, the raw materials and the final product specification is the first step in building a model. After that, the necessary data must be collected either from your own experiments (preferred) or external sources (often used to fill the own data gaps and also to validate your own experiments). Following this, it is necessary to define the reactions in a process and its parameters such as yields, reaction (fermentation) time, product concentration, by-product formation, etc. In the next step, the process flow diagram, unit operation and process streams are defined, and finally, the simulation is performed and an analysis of the results made. Usually, before achieving final results several of those steps are repeated and improved. (Heinzle et al., 2006; Towler and Sinnott, 2008)

4.2. On-farm energy production

Biomass is a key parameter in an agriculture environment for energy production (Jørgensen et al., 2005); two of the very promising technologies which could be applied directly on the organic farm are: anaerobic digestion for production of biogas and ethanol fermentation (Frederiksson et al., 2006). Throughout this study process models for a single organic farm (around 100 ha) (**Paper VI**) and several organic farms (around 1000 ha) were developed (**Paper VII**). This biorefinery consists of two processes: ethanol fermentation and anaerobic digestion (Figure 13).

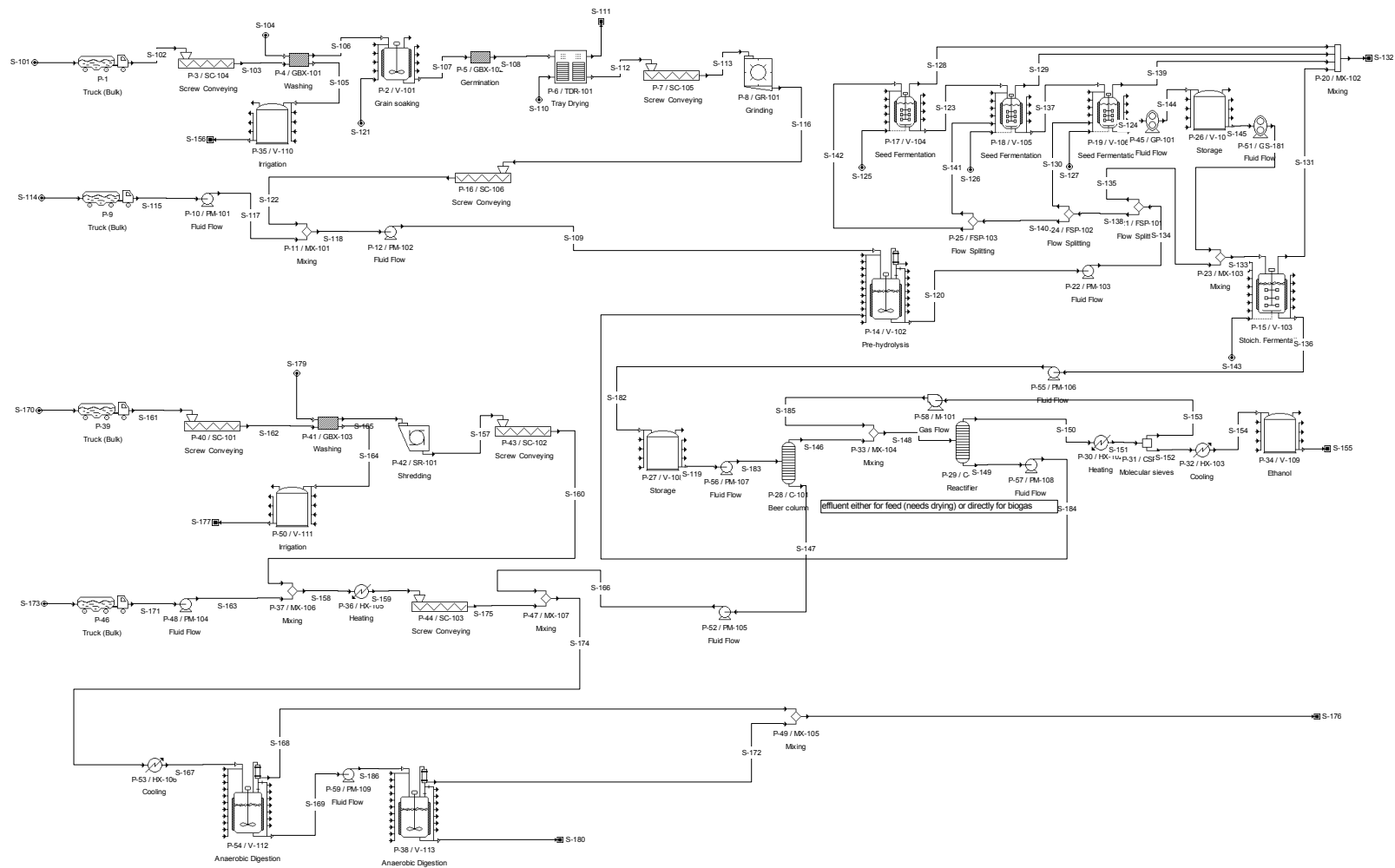


Figure 13. The on-farm biorefinery for co-production of ethanol and biogas (flow sheet from SuperPro Designer)

Ethanol is produced from rye grains and whey. Rye grains are soaked with water prior to germination to achieve moisture of 40-45%. Germination takes 24 hours at room temperature (25°C), during that process, natural amylases are produced. After germination, the grains are dried at 35°C, grinded and then mixed with whey. Germinated grains contain sugars, whereas whey supplies the process with nutrients, process water and an additional carbon source (lactose). To activate the enzymes, the input stream is pre-hydrolyzed at 50°C. The fermentation is carried out by *Kluyveromyces marxianus* at 40°C with a hydraulic retention time of 40 hours. Inoculum can be either produced in 3 step seed fermentors directly on the farm or bought from outside. Finally, the ethanol is purified in a two step distillation and the remaining water removed in molecular sieve. A final ethanol concentration of 99.6% is achieved.

Biogas is produced from cattle manure, clover grass and maize silages (**Paper VI**) or cattle manure and clover grass silage (**Paper VII**). Crops, after harvesting, are stored in silage form (ensiling process was not included in a model); both are shredded before being added to the fermentor where they are mixed with cattle manure in a ratio depending on the scenario (scenarios are described in detail in **Paper VI** and **VII**). The anaerobic digestion process is performed in two stage continuous mode at thermophilic conditions (55°C) with a hydraulic retention time of 20 days with each reactor. During the process, biogas with a methane content of 60-65% is collected. The effluent from the anaerobic digestion (digestate) is commonly used as fertilizer. It contains undigested lignocellulosic leftovers (valuable carbon source for soil) and significant amounts of nitrogen, phosphorous and potassium - all originating from manure.

Several authors studied the possibility of energy production on a farm scale level. Table 2 summarizes those works showing raw materials, technology applied and presents main conclusions. It is suggested that the popular technologies - biogas and rape methyl ester - could be produced directly on the farm (Svesson et al., 2005; Svesson et al., 2006; Frederiksson et al., 2006; Hansson et al., 2007; Monreal et al., 2007;) Ahlgren et al (2008; 2009) suggested Fisher-Tropsch fuel or thermo-chemical gasification products. All the authors mentioned their concerns about the expensive downstream processing to clean the fuel (e.g. biogas upgrading or ethanol distillation) or the necessary engines modification to adjust to new fuels.

Table 2. Different on-farm bioenergy production concepts

Raw materials	Technology/ Fuel	Main Conclusions	Reference
Short rotation coppice (SRC)	-	5% of agricultural land could produce energy equaling 30-58% of the energy input for organic farming; Utilization of wastewater and sewage sludge to close the gap between agriculture and the cities; SRC crops reduce nitrate leaching (protection of water quality); SRC fields could be an outdoor areas for e.g. pig and poultry;	Jørgensen et al., 2005
Sugar beet tops, wheat straw, ley crops	Biogas	Technology: single stage fed-batch high-solids digestion; The results indicated the importance of choosing a substrate with a high methane yield and high N content; Positive effect of scale was observed, Positive effect of economy of numbers was discussed (significantly decreasing cost);	Svesson et al., 2005; 2006
Winter rapeseed	Rape methyl ester (RME)	Favorable energy balance; High land use and the emission associated with cultivation; Well-known technology and easy to implement on the farm scale; 72% reduction in GHG when compared to diesel;	Frederiksson et al., 2006
Winter wheat (grains)	Ethanol	Energy consuming process; Low area needed for cultivation; Technology is available but it is more optimal for large scale; 60% reduction in GHG compared to diesel;	
Silage	Biogas	Low need for arable land (small emission of GHG); Advantageous recycling of plant nutrients; Small scale technology for biogas upgrading is not optimal; 58% reduction in GHG compared to diesel;	
		Fuels produced outside the organic farm in industrial scale plants;	Hansson et al., 2007

		Systems based on the production of one raw material but with access to different fuels are economically favorable;	
Rapeseed	Rape methyl ester	8.5% of the farm land is needed to achieve self-sufficiency in motor fuel; The total energy efficiency (energy in the fuel/total allocated energy use): 8.3; No engine modification needed; High price of the organically produced rapeseed;	
Wheat	Ethanol	5.5% of the farm land is needed to achieve self-sufficiency in motor fuel; The total energy efficiency (energy in the fuel/total allocated energy use): 2.6; Low cost in large production facilities;	
Ley	Biogas	3.8% of the farm land is needed to achieve self-sufficiency in motor fuel; The total energy efficiency (energy in the fuel/total allocated energy use): 4.4; Raw materials available in large amounts; Cost of transport, storing and cleaning the gas is high; Significant modification in engine is needed if run only on gas;	
Animal manure/ straw and sorted municipal wastes	Biogas/ Gasification	5 different projects running for on-farm renewable energy production and GHG mitigation: Effective use of produced electricity with surplus fed to the grid, produced heat used for digester heating only; Planned nutrients recovery and concentration from AD effluent to produce 'nutrient-rich bio-fertilizer' was planned;	Monreal et al., 2007
Straw, short rotation willow coppice (Salix)	Fisher-Tropsch diesel (FTD)	To achieve self-sufficiency, 108 ha of Salix and 261 ha of straw collected (no land set aside) is needed out of 1000ha; Energy balance 8.9 and 9.6 from Salix or straw; Additionally, large amount of by-products is produced;	Ahlgren et al., 2008

	Dimethyl ether (DME)	To achieve self-sufficiency, 38 ha of Salix and 70 ha of straw collected (no land set aside) is needed out of 1000ha; Energy balance 10.1 and 10.0 from Salix or straw;	Ahlgren et al., 2009
		Fuel produced outside of the farm, utilized in fuel cell powered tractors; Studied technologies are not yet on a commercial scale and available at reasonable costs	
Straw	Hydrogen - thermochemical gasification	To achieve farm self-sufficiency, no land is needed to be set aside, but straw collected from 43 ha (out of 1000 ha); Energy balance 16.3; 97% reduction in GHG when compared to diesel;	
Straw	Methanol - thermochemical gasification	To achieve farm self-sufficiency, no land is needed to be set aside, but straw collected from 53 ha (out of 1000 ha); Energy balance 19.5; 97% reduction in GHG when compared to diesel;	
Salix	Hydrogen - thermochemical gasification	To achieve farm self-sufficiency, 16ha (out of 1000 ha) is required; Energy balance 14.2; 92% reduction in GHG when compared to diesel;	
Salix	Methanol - thermochemical gasification	To achieve farm self-sufficiency, 20ha (out of 1000 ha) is required; Energy balance 15.6; 91% reduction in GHG when compared to diesel;	
Ley	Hydrogen – biogas production	To achieve farm self-sufficiency, no land is needed to be set aside, but green manure harvested from 43ha (out of 1000 ha); Energy balance 6.1; 90% reduction in GHG when compared to diesel;	

In **Paper VI**, the scenario for energy sufficiency at the 100 ha organic farm was discussed. Two scenarios were considered: biogas and bioethanol production. The organic farm energy requirement was estimated at 180 GJ. According to (Frederiksson et al., 2006; Hansson et al., 2007) to produce 1 MJ of biogas, 216 kJ is needed, and to produce 1 MJ of ethanol, 228 kJ. The overall organic farm energy requirement accounted those values. The efficiency of CHP unit was estimated at 38%. Based on those assumptions it was concluded that 16.2% of the farm land area is need to produced ethanol from rye grains, or 8.2% of the farm area to generate biogas from maize and clover grass silages to achieve.

Hansson et al., (2007) indicated that only 5.5% of the farm area is need to produce a sufficient amount of ethanol and 3.8% for biogas to substitute motor fuel. To supply the organic farm with Fisher-Tropsh diesel or dimethyl ether, according to Ahlgren et al. (2009) 3.8 – 10.8% of farm land is necessary (Salix plantation). If fuel cell technologies are applied and a thermo-gasification product (such as hydrogen or methanol) or hydrogen produced from biogas, much less farm land is necessary to fulfill tractive power demands. It was indicated that less than 5% of the farm would be required. However, all of those technologies are still under development and the study considered only hypothetical production.

In **Paper VII**, scenarios to produce renewable energy at the farm were simulated. The diagrams describing them are presented on Figure 14. Five cases were designed to meet possible potentials of an organic farm: production of biogas from clover grass silage and cattle manure (two scenarios), production of bioethanol from rye grains and whey (two scenarios), and the combination of those two to produce on-farm biogas and bioethanol.

The crop distribution, number of animals and amount of manure in the baseline (Figure 2) are based on the statistical data on organic farmers in Denmark in 2006. The area for each crop, number of animals and amount of manure are means of full time organic farmers on sandy soil, being either dairy farmers or cash crop farmers. This farm type represents 61 % of organic farmers in Denmark. The data used origins from the Single Payment applications of Danish farmers. In 20% of the scenarios, the number of dairy cows are reduced in order to make room for a larger bioenergy production. Therefore, less manure is available. (Pugesgaard et al., in preparation)

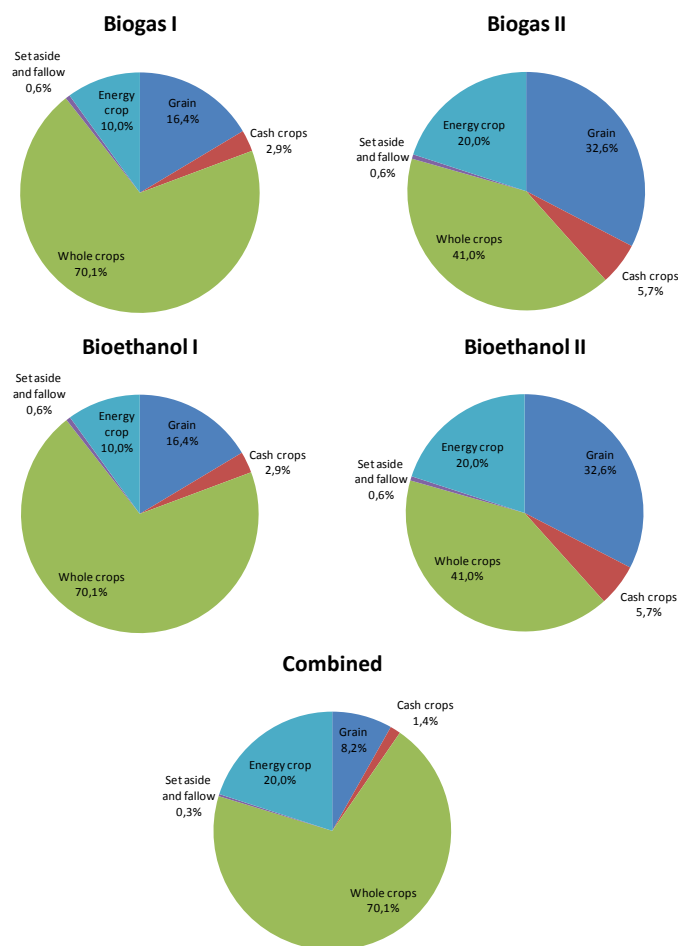


Figure 14. Five scenarios for bioenergy production at 1000ha organic farm

The main results from the study are shown in the Figure 15. The combined scenario was characterized by the highest investment but also by the largest energy produced (29244 GJ/year). Scenario Biogas II was second best in terms of the amount of produced energy (26409 GJ/year) and it was characterized by slightly lower investment cost compared to the scenario Biogas I, which was also less effective in terms of energy (19970 GJ/year). Although, Bioethanol I and Bioethanol II presented the lowest investment costs, they also generated the least energy (4034 GJ/year – Bioethanol I and 5610 GJ/year – Bioethanol II). Scenario Bioethanol I indicated a slightly higher total capital investment compared to the scenario Bioethanol II. Bioethanol scenarios include downstream processing (distillation) which increase the total fuel production cost, in case of the Biogas scenario, upgrading biogas to natural gas quality was found not to be necessary.

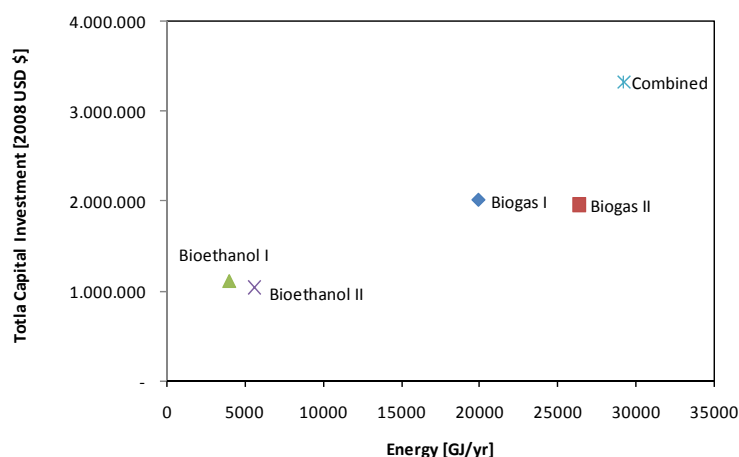


Figure 15. Relationship between total capital investment of each scenario and produced energy (in GJ/yr)

The achieved results for the Biogas scenarios correspond to what was calculated in a Danish report for the development of on-farm organic biogas plant (Tersbøl and Jørgensen, 2009). The Ethanol scenarios built were based on large scale ethanol industry (Wooley et al., 1999; Aden et al., 2002; Klein-Marcuschamer et al., 2010). Therefore, it is believed that those are also reliable. All the assumptions in building this model were described in **Paper VII**. That work presented an engineering tool which could be used in organic farming community to design and evaluate economic feasibility of an on-farm organic biorefinery. A large range of scenarios with different process configurations suitable for specific organic farm could be simulated and best one chosen.

4.3. Lignocellulosic biorefinery

The biorefinery refines and converts biological raw materials (biomass) into multiple valuable products (Kamm and Kamm, 2004). Similarly to the petroleum refinery, the biorefinery should produce several different industrial products such as: transportation fuels, commodity chemicals and materials as well as high-value, low-volume speciality chemicals. At the moment, energy is a precursor and driver in this development, but over time other more sophisticated products will be developed (Clark and Deswarte, 2008). An example of the basic scheme of biorefinery is shown in Figure 16.

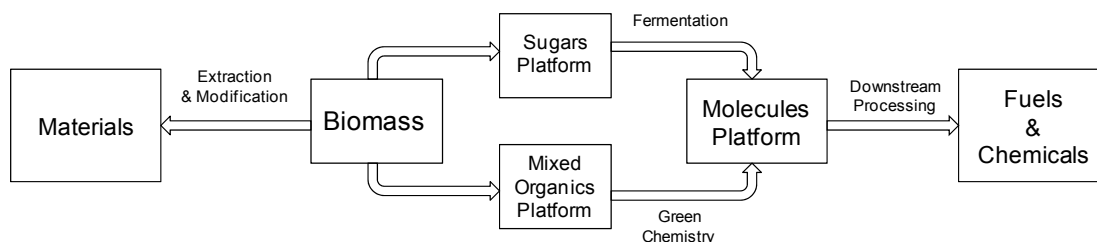


Figure 16. Biorefinery (adapted from Clark, 2007)

Biomass (seen as a platform) can be split into sugars and mixed organics platforms from where, through biological or chemical routes, a wide range of molecules are created. Apart from that, directly from biomass, valuable products can be fractionated through extraction and modification or thermochemical processes as well as traditional chemical methods. By further processing, it is turned into fuels and high value products.

The principal rule of biorefinery should be to maximize the value of the biomass and minimize waste. In other words, all the streams should be utilized and converted into useful components. Costs will be cut down if the used fraction of biomass is increased.

Usually biorefinery products are diluted in complex aqueous solutions (e.g. ethanol in the fermentation broth). It is desired to make downstream processing, which typically is an expensive and wasteful stage of the process, a clean and low energy technique that could convert multicomponent systems into valuable clean products.

Detailed schemes on possible products from biorefinery is shown in Figure 17. Building blocks based on single to six carbon chemical compounds, aromatics or direct polymers are possible. Generally, a wide range of products for industry, transportation, housing, health purposes etc. may be produced. Modern biorefineries should follow market needs and be easily adjustable to produce, besides bulk chemicals and energy which would be the core of the plant, low-volume high-value chemicals filling market gaps. Additionally, biorefineries should be able to use various types of feedstocks - that way it can adapt towards changes in demand and supply for feed, food and industrial commodities (Kamm and Kamm, 2004).

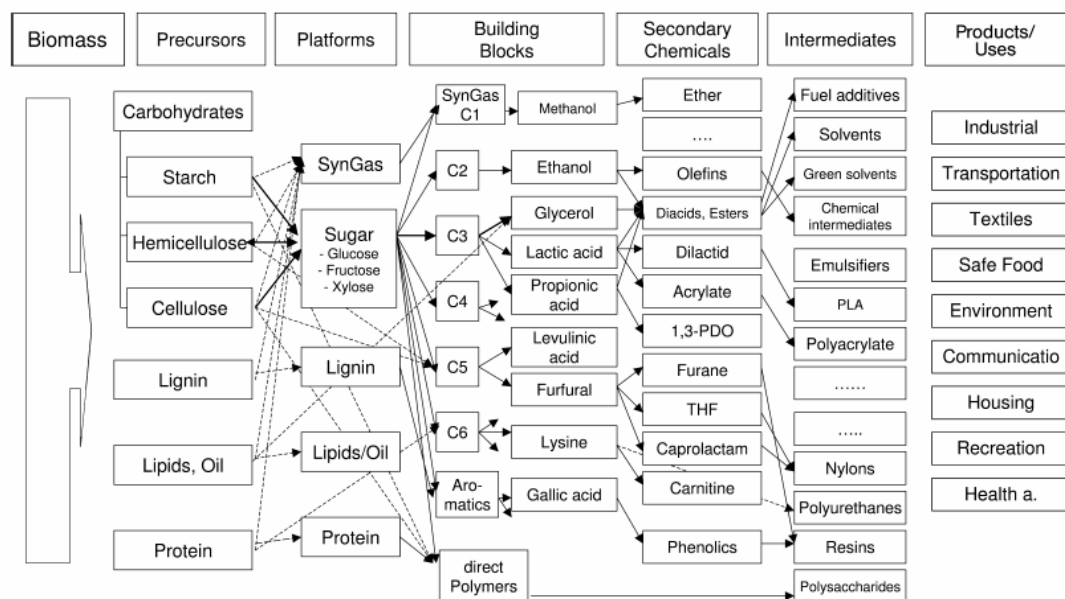


Figure 17. Model of a biobased products flow-chart for biomass feedstock (Werpy and Petersen, 2004)

4.3.1. Techno-economic models

Several techno-economic models about the potential of biofuels were published (Wingren et al., 2003; Aden and Foust, 2009; Sotoft et al., 2010). Many of them are limited to a set of scenarios and naturally cannot meet all the possible options which would be beneficial for broad biofuels community. The created techno-economic model of a lignocellulosic ethanol biorefinery presented in **Paper VIII** comes towards those needs. The model is deposited online and is available for download and evaluation; this tool can be revised by the academic and professional research community.

Scheme of lignocellulosic ethanol biorefinery is shown in the Figure 18, a detailed description of the whole process is available in **Paper VIII**. Apart from the base case, several scenarios were modeled: reducing acetate content of the biomass feedstock, increasing cellulolytic enzyme activity, reducing lignin content of the biomass content, increasing the rate of xylose-fermentation by yeast, and increasing the tolerance of yeast to acetic acid and ethanol. The total capital investment cost for all investigated scenarios (for modeled facility, which treats 2000 tons/day of wet biomass (app. 85% dry matter)) vary between 315 and 370 MM USD\$.

The aim of the study was to develop a dynamic modeling tool through which different research groups, focusing on several stages in the biorefinery process, could communicate. In that way, full techno-economic model would be created, bringing the economical and environmentally sustainable bioproducts closer (in this case liquid biofuels).

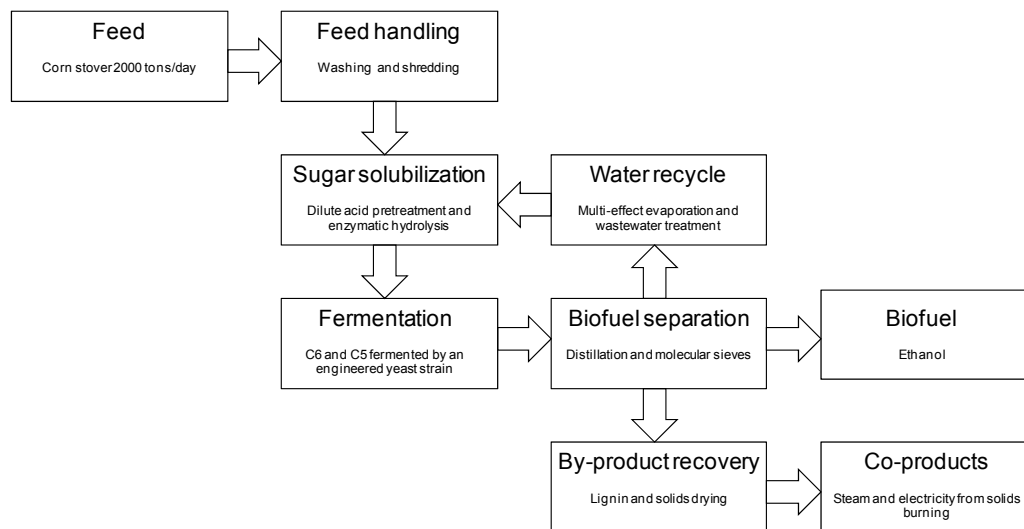


Figure 18. Scheme of lignocellulosic ethanol biorefinery (adapted from **Paper VIII**)

In **Papers VII** and **VIII**, models for two concepts of the biorefineries were discussed: the small farm scale, dedicated to develop self-sufficiency in the energy supply for an organic farm and the large industrial scale which aims for the bulk production of biofuels (bioproducts). The optimal size of biorefinery is not known yet, however, Clark and Deswarte (2008) believe that it should be a combination of large scale facilities - which would be advantageous for both economy of scale, and small scale plants - which could keep the transport cost to minimum by preprocessing and concentrating biomass or intermediate products (Clark and Deswarte, 2008). According to (Realff and Abbas, 2004) the goal is to find the balance between the increasing cost of transportation low-yield material and the reduction in costs in the increasing the scale of the process. Hess et al. (2003) indicated that to develop a sustainable biorefinery it is crucial to reduce the cost of collection, transportation and storage of biomass; it can be done through densification of raw material. Local small scale pretreatment/preprocessing units will play a significant role in a successful, economically feasible biomass based refinery. Realff and Abbas (2004) discussed that in some cases beneficial can be intermediate concentration of the biomass into a liquid form and then transport to a larger biorefinery. This will also allow farmers to play an important role in the supply chain, not only in biomass cultivation but also in the processing part.

5. Concluding remarks

In the work presented in this thesis, the possibility of biogas and bioethanol production in organic farming was investigated. The idea was evaluated from choosing and estimating the potential of raw materials through continuous trials on biogas and ethanol production, the investigation of simplified pretreatment method for lignocellulosic materials, and finally, the simulation of on-farm biorefinery concept and development of an open modeling tool for a lignocellulosic ethanol biorefinery.

The methane and ethanol potential from selected raw materials (maize, rye, clover, vetch, cattle manure and whey) were measured and evaluated. All the investigated substrates were suitable for energy production, either through anaerobic digestion or yeast fermentation. Maize and rye were characterized by the highest potential, both for methane production. During continuous trials, a significant boost of methane production was read after the increased ratio of maize silage to cattle manure in feed, and no inhibition of the process was noticed. The ensiling method was assessed for its suitability for lignocellulosic ethanol production. Maize, rye, clover and corn stover were ensiled in laboratory conditions and used as substrates in yeast fermentation. Very promising results were achieved, concluding that ensiling is a very efficient wet-storage method or even biological pretreatment method for second generation ethanol. Moreover, ethanol production by *K.marxianus* from organic whey in continuous process resulted in high ethanol productivity where neither sterilization nor pasteurization was needed. The experiments proved that whey is a suitable medium for ethanol production and successfully could be used in the on-farm biorefinery concept. Based on the results from laboratory experiments and additional literature data, simulation models for on-farm bioenergy production was built. The first results validated that there is enough land on the farm to supply it with self-produced energy. Further investigation led to the development of a techno-economic model where five scenarios were evaluated for on-farm energy generation. The aim of the presented modeling tools, both for small or industrial scale biorefineries, were to build a platform for differently sized biorefineries. The simulation models can be edited and adjusted to the specific needs. This way, the on-farm bioenergy production as well as large scale lignocellulosic biorefineries can be brought closer to the reality.

6. Future outlook

The development of on-farm biorefineries requires a further update and optimization of the processes involved and building and adjusting simulation models, which can help the successful establishment of such a facility if required for particular needs. Several configurations should be modeled and the most suitable chosen. These configurations might differ from each other depending on the specific farm requirements. New process solutions such as ensiling method (presented in this thesis) and modern fuels (Ahlgren et al., 2009) or new generation fuels which are still in a lab phase (Steen et al., 2010) might be the answers of the future. Additionally, constructing more facilities, more on-farm biorefinery plants, will surely lead to a price drop, similar to the one experienced during development of the biogas plant in Denmark in 80s and 90s (Mæng et al., 1999).

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List of papers

Paper I: Oleskowicz-Popiel P., Thomsen M.H., Nielsen H.B., Schmidt J.E., Thomsen A.B.: Characterization of most relevant feedstock for biogas and bioethanol production in organic farming. Vth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunisia, 25-28 May, 2008.

Paper II: Oleskowicz-Popiel P., Nielsen H.B., Thomsen A.B., Schmidt J.E.: Biogas and ethanol potentials in selected biomasses for organic farming. *Submitted*

Paper III: Oleskowicz-Popiel P., Schmidt J.E., Thomsen A.B.: Ensiling – a wet-storage and a biological pretreatment method for bio-ethanol production from lignocellulosic biomasses. *Submitted*

Paper IV: Oleskowicz-Popiel P., Lehtinen T.M., Schmidt J.E., Thomsen A.B.: Ensiling – wet-storage and pretreatment of corn stover to produce bioethanol. *Submitted*

Paper V: Christensen A.D., Kadar Z., Oleskowicz-Popiel P., Thomsen M.H.: Production of bioethanol from organic whey using *Kluyveromyces marxianus*. Journal of Industrial Microbiology and Biotechnology, 2010, DOI 10.1007/s10295-010-0771-0.

Paper VI: Oleskowicz-Popiel P., Thomsen M.H., Thomsen A.B., Schmidt J.E.: A simulation model of combined biogas, bioethanol and protein fodder co-production in organic farming. International Journal of Chemical Reactor Engineering, 2009, vol.7, Article A71.

Paper VII: Oleskowicz-Popiel P., Schmidt J.E.: Techno-economic analysis of bioethanol and biogas production in organic farming. *Proceeding submitted to 12th World Congress on Anaerobic Digestion*, Guadalajara, Mexico, October 31st – November 4th, 2010.

Paper VIII: Klein-Marcuschamer D., Oleskowicz-Popiel P., Simmons B.A., Blanch H.W.: Techno-economic analysis of biofuels: a wiki-based platform for lignocellulosic biorefineries. Biomass and Bioenergy, 2010, doi:10.1016/j.biombioe.2010.07.033.

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